https://doi.org/10.56946/jspae.v2i1.180

ISSN: 2957-9082
Journal of Soil, Plant and Environment

ORIGINAL RESEARCH

Integrated Effect of Heavy Metal-Tolerant Rhizobacteria and Phosphorus on Maize Growth and Phosphorus Bioavailability in Contaminated Soil

Aman Nawaz[1*], Haq Nawaz[1], Kamal Khan[1], Mauz ul Haq[1], Hassan Khan[1], Umair Mananman[1], Muhammad Tariq[1]

[1]Department of Soil and Environmental Sciences Faculty of Crop Production Sciences, The University of Agriculture, Peshawar-Pakistan
*corresponding author: soilscience810@gmail.com

Received: 01 Oct 2022
Revised: 03 March 2023
Accepted: 03 June 2023

ABSTRACT: Toxic metals significantly influence agricultural crop yields. Hence, in the current study, the impact of different strains of phosphorus-solubilizing bacteria (PSB) and levels of phosphorus on the solubility of phosphorus and the stabilization of heavy metals in contaminated soil were studied in a greenhouse pot experiment in 2022. The PSB strains included *Bacillus CUM6*, *Bacillus DUM7*, and *Bacillus PIS7*. Phosphorus was applied at rates of 40, 60, and 80 kg P_2O_5 ha^{-1} using single superphosphate, with a basal dose of 120 kg N and 60 kg K_2O ha^{-1}. Maize plants (variety "Azam") were inoculated with the PSB strains. Consequently, combining *Bacillus PIS7* with 80 kg P_2O_5 ha^{-1} significantly increased fresh and dry maize biomass (35.33 and 24.56 g pot^{-1}) compared to the control (unspiked soil). Phosphorus bioavailability (7.07 and 5.14 mg kg^{-1}) significantly improved with 80 kg ha^{-1} phosphorus and *Bacillus PIS7*. Heavy metal concentrations in soil (Cd, Pb, Cr, and Ni) decreased significantly at 80 kg ha^{-1} phosphorus, and *Bacillus PIS7*, and Cd concentrations in plants decreased to 3.31 mg kg^{-1} with *Bacillus DUM7* and to 2.96, 0.42, and 1.33 mg kg^{-1} with *Bacillus PIS7*. The application of PSB strains and phosphorus fertilizer reduced heavy metal concentrations. Notably, 80 kg P_2O_5 ha^{-1} with *Bacillus PIS7* showed the best performance. Phosphorus uptake increased significantly (0.106 mg pot^{-1}), while heavy metal uptake (Cd, Pb, and Cr) decreased linearly with increasing phosphorus levels and PSB strains. *Bacillus PIS7* with 80 kg P_2O_5 ha^{-1} had the lowest heavy metal translocation, doing better than the control and other PSB strains (*Bacillus CUM6* and *Bacillus DUM7*). Soil characteristics indicated increased organic matter content (0.73%) and decreased pH (7.61) and electrical conductivity (0.17 dSm^{-1}) with applied phosphorus and PSB strains, suggesting enhanced phosphorus bioavailability and reduced heavy metal concentrations. In conclusion, adding 80 kg P_2O_5 ha^{-1} with *Bacillus PIS7* is recommended to achieve better growth response under heavy metals stress andstabilizen soil.

KEYWORDS: Maize, heavy metals, phosphorus solubilizing bacteria electrical conductivity, Biomass

1. Introduction

Toxic metals have been widely disseminated and are the major source of concern for agricultural sustainability and living standards (Kesari et al., 2021). Fertilizers, industrial effluent, pharmaceuticals, mines, wastewater, and other human activities may release them into the ecosystem (Manasa et al., 2020). There is unquestionably a pressing need to boost agricultural productivity by removing metals poisons from the food chain (Alsafran et al.,

2023). If there is a high concentration of heavy metals in the growing media, plants, aquatic life, and human health can all be adversely affected (Sardar et al., 2013). Heavy metals harm soil biota by harming important microbes and reducing the quantity and activity of soil microorganisms (Kamal et al., 2010).

Soil remediation is becoming difficult due to technological and financial constraints (Khalid et al., 2017). Different biological, physical, and biochemical techniques have been explored to attain this aim during the last several decades (Sheoran et al., 2011; Wuana and Okieimen, 2011). These methods have several drawbacks, including extensive work, high costs, disruption of native soil microorganisms, and long-term changes in soil characteristics. Phosphorus solubilizing bacteria (PSB) which promote plant growth in a metal laminated media have lately been researched for their capacity to promote plant development and production while reducing the uptake and bioavailability of heavy metals to plants. The phosphate solubilizing bacteria are extremely effective in immobilizing heavy metals and inhibiting their redistribution in crops through precipitating, binding affinity and sorption (Xu et al., 2019). The combination of plant growth enhancing rhizosphere strains P. aeruginosa and B. gladioli in soils increased plant development in terms of root growth, stem length, and fresh and dry weights in *Esculentum* L. seedlings subjected to Cd stress (Khanna et al., 2018). Cd exposure microbe inoculation seeds showed a significant improvement in photosynthesis rate by boosting the amount of photosynthetic pigments such as chlorophyll, carotenoid, and xanthophylls (Khanna et al., 2019).

The phosphate solubilizing bacteria play a crucial mechanism in enhancing plant growth and yield. Bacteria have an important role in the transformation, bioavailability, and breakdown of organic materials, all of which are efficient in extracting P from both inorganic and organic sources (Mengoni et al., 2001; Delorme et al., 2001). Due to unintentional metal ion absorption, metal pollution affects plants, bacteria, and primary producers (Rizvi et al., 2022). Using metal hyperaccumulator plants, phytoremediation improves efficiency by actively extracting and accumulating metals (Yan et al., 2020). Enhancing the rhizobiome interaction can overcome metal uptake restrictions, aiding metal availability and plant growth under various stressors (Rizvi et al., 2022). In situ, solutions reduce costs and address pollution dispersion challenges. However, phytoremediation has drawbacks like phytotoxicity, slower action, and limited absorption. Certain plant-associated bacteria mitigate these issues and enhance phytoremediation, especially endophytes that reside inside plants without harming them (Feng et al., 2017). This symbiotic relationship improves plant resistance to stressors in exchange for carbohydrates from endophytes.

Hence, the simultaneous application of phosphate-solubilizing bacteria (PSB) strains and phosphorous can significantly positively affect plant growth in stressful environments (Dey et al., 2021). Furthermore, the combined use of PSB strains and phosphorous fertilizer has the potential to significantly reduce the concentration of heavy metals such as Cd, Pb, Cr, and Ni in both soil and plant shoots and

roots (Xu et al., 2019). Additionally, this approach can enhance the bioavailability of phosphorous and improve its uptake by plants. Therefore, a pot experiment was conducted using the above mentioned theme with the following main objectives: (1) To assess the impact of different PSB strains and P levels on P bioavailability to maize. (2) To assess the impact of different PSB strains and P levels on the growth of maize under heavy metals stress conditions. (3) To assess the impact of different PSB strains and P levels on the bioavailability of heavy metals stress conditions. (4) To assess the impact of different PSB strains and P levels on plants' P and heavy metals uptake and phytoremediation of heavy metals stress in soil. Through this comprehensive study, we aim to shed light on the potential benefits of utilizing specific PSB strains and phosphorous in enhancing plant growth and mitigating the effects of heavy metals stress. The results of this research will contribute to the development of sustainable strategies for improving soil quality and plant productivity in contaminated environments.

2. Materials and Methods

2.1 Experimental Description

The soil for the pot experiment was collected from The University of Agriculture Peshawar's Research Farm. After removing plant materials, pebbles, stones, and plastic garbage from the soil, a bulk sample was collected using a clean spade. Once dry, the soil was sieved through a 2 mm screen. Each pot was filled with 10 kg of the sieved soil. Finally, all pots were spiked with heavy metals (Cd, Pb, Cr, Ni) at a concentration of 10 mg kg^{-1}. Maize variety Azam seeds were sterilized and inoculated with PSB inoculate containing 1.5 × 107 CFU of PSB g^{-1}. For inoculation, 50 g of sugar-soaked maize seeds were treated with 8 g of PSB inoculum (2 kg PSB inoculant 25 kg^{-1} seeds ha^{-1}) following the method used by Alagawadi and Gaur (1998). PSB strains included Bacillus CUM6, Bacillus DUM7, and Bacillus PIS7, while phosphorus was applied at rates of 40, 60, and 80 kg P_2O_5 ha^{-1} as single superphosphate (SSP), with a basal dose of N 120 and K_2O kg ha^{-1} as urea and sulfate of potash (K_2SO_4), respectively. Maize seeds were infected with PSB using a standard inoculation process as recommended.

2.2 Soil Sampling and Analysis

The soil samples were collected soon after the harvesting, air dried and sieved through a mesh size of 2 mm to measure soil chemical properties. The pH of the soil was determined using the Thoms, (1996) method.

The organic matter in the soil was determined using the Nelson and Sommers (1996) method. Prior to analyzing the sample, a blank sample was run to ensure the accuracy and precision of the chemicals used in estimating the organic matter content in the soil using the method in Equation 1.

$$SOM\ \% = \frac{(\text{ml of} K_2Cr_2O_7 \text{ x N}) - (\text{ml of } FeSO_4.7H2O \text{ x N})}{\text{weight of soil (g)}} \times 0.69$$

-Eq-1

The Bremner and Mulvaney technique (1996) was used to calculate the amount of total nitrogen present in the soil. In this procedure, 0.2 g of soil, 1.1 g of digestion mixture, and 3 mL of concentrated H_2SO_4 were all added to a digestion tube. The digestion tube was kept in a fume hood on a

digestion rack for five hours. The suspension was transferred to a 100 mL plastic bottle after digestion was complete, a small amount of distilled water was added, and the bottle's capacity was extended to 100 by adding distilled water.

$$\text{Soil N } (g\ Kg^{-1}) = \frac{(s-B) \text{ x meq. N x N of Hcl x volume made}}{\text{Weight of soil (g) x volume for distillation}} X \frac{1000\ g}{1\ Kg}$$
Eq-2

Phosphorus content in the soil was determined using the AB-DTPA extraction method developed by Soltanpour and Schwab (1977). After color development, the soil samples were analyzed using a spectrophotometer, and the readings were recorded.

$$AB-DTPA \text{ extractable P } (mg\ kg^{-1}) = \frac{\text{Instrumental readings X Volume made}}{\text{Weight of soil}}$$
Eq-3

The Soltanpour and Schwab (1977) method was employed to determine the concentrations of Cd, Pb, Cr, and Ni in the soil. The resulting solution was then filtered through Whatman-42 filter paper into a clean 100 mL plastic bottle. A spectrophotometer equipped with atomic absorption capability was utilized to measure the levels of the respective heavy metals (Equation-4).

$$\text{Cd, Pb, Cr, Ni } (mg\ kg^{-1}) = \frac{\text{Instrumental readings X Volume made}}{\text{Weight of soil}}$$
-Eq-4

2.3 Plant sampling and analysis

In the pot experiment, after the designated period, the entire plants were carefully harvested by cutting them at the soil surface. The harvested plants were then transported to the laboratory for further analysis. To ensure accurate measurements, the plant samples were subjected to air drying to remove any moisture content. Once completely dried, the plant biomass was measured using a digital balance. In addition to measuring biomass, the plant samples were also analyzed for nutrient content, including phosphorus uptake and heavy metals.

The method of wet acid digestion was used to determine the amount of phosphorus in the plant (Fagbenro et al., 1998). In this procedure, 0.5 g of oven-dried plant material is placed in a flask with 10 mL of HNO_3 and left on a table overnight. The plant sample received 4 mL of $HCLO_4$ in addition, and it was held on a hot plate until it started to boil. When white vapors started to appear, the process was complete, and the flask was taken off the hot plate. After chilling, the volume was made up to 100 mL. In order to alter color, 0.5 mL of the plant sample was added to a 25 mL volumetric flask containing 5 mL ascorbic acid. The volume was then made up to the mark with distilled water. The flasks were then stored in a dark area for 5 minutes. A spectrophotometer was used to calculate the amount of P present in the plant sample. The apparatus was calibrated using standard solutions of phosphorus at various concentrations before processing the plant samples.

The AOAC method of wet acid digestion was used to determine Cd, Pb, Cr and Ni in plants (Zheljazkov et al., 2008). The plant materials were air dried first, then placed for 48 hours in a 70°C oven drying. The root and shoot samples were pulverized on a grinder after drying. A 0.25 g of plant material was placed into a conical flask that held 250 mL of HNO_3 and was left overnight to continue the procedure. After 24 hours, 4 mL of

$HClO_4$ was added, and the flask was heated on a hot plate until the sample turned clear. The flask was cleaned with distilled water, filled to 100 mL, and placed in a spotless plastic container for storage. The levels of heavy metals in plant shoots and roots were measured using an atomic absorption spectrophotometer. Prior to employing plant samples for Cd, Pb, Cr, and Ni contents, the machine's accuracy was evaluated using reference solutions of the appropriate heavy metals.

2.4 Secondary parameters

The bioaccumulation and translocation factors were calculated according to Muhammad et al. (2020).

P uptake=P content in Plant × dry matter yield /100

The total uptake of P and all the heavy metals were calculated by multiplying their shoot concentrations with plant shoot dry weight and then divided by 100.

Translocation Factor= Contents in (Shoot/Root)

2.5 Statistical analysis

Complete randomization was used in the experiment's design. The Stastix 8.1 package was used to analyses the data. At a significance threshold of 0.05, the least significant difference test (LSD) was applied to the significant data. Data with substantial variations were given different alphabetic letters (Steel et al., 1997).

3. Results and discussion

3.1 Physio-chemical properties of experimental soil prior to the experiment

Prior to the experiment, composite soil samples were collected to evaluate the test soil's physiochemical properties (Table 1). The soil was silt loam in texture, the alkaline in reaction, non-saline in nature, moderately calcareous, low in organic matter content, moderate in AB-DTPA extractable P (Soltanpour and Schwab 1977) and AB-DTPA extractable heavy metals (Cd, Pb, Cr and Ni) were found below the permissible limits (Tóth et al., 2016).

Table 1. Physiochemical properties of the test soil prior to experiment.

Soil Properties	Values
Sand	21.8%
Silt	67.3%
Clay	10.9%
Textural class	Silt loam
pH $_{(1:5)}$	7.72
EC $_{(1:5)}$	0.19
Organic matter	0.51%
Lime	12.50%
Total N	0.057%
AB-DTPA extractable P	4.36 (mg kg^{-1})
AB-DTPA extractable Cd	0.04 (mg kg^{-1})
AB-DTPA extractable Pb	3.31 (mg kg^{-1})
AB-DTPA extractable Cr	0.42 (mg kg^{-1})
AB-DTPA extractable Ni	0.41(mg kg^{-1})

3.2 Effect of rhizobacteria and applied phosphorous on the plant growth response under heavy metals stress environment

3.2.1 Plant height (cm)

Maize plant height was significantly affected by phosphorous solubilizing bacteria (PSB) in conjunction with various levels of phosphorus (P) (Table 2). A taller plant of 112.50 cm was recorded in Bacillus *PIS7*, followed by *Bacillus DUM7* with a height of 109.25 cm compared to other PSB stains. Among the various P levels, the maximum plant height of 112.33 cm was achieved at a

rate of 80 kg ha^{-1}. Notably, the highest plant height of 122 cm was observed when Bacillus PIS7 was combined with 80 kg P ha^{-1}. This was closely followed by Bacillus DUM7 with 80 kg P ha^{-1}, resulting in a plant height of 117 cm. Conversely, Bacillus CUM6 without P application displayed the lowest plant height of 91 cm. Optimal Cd concentrations in soil significantly decreased plant height (Zafar-ul-Hye et al., 2020). The application of phosphorus enhances plant growth parameters, such as plant height, under Cd stress conditions. (Naeem et al., 2018; Ao et al., 2014; Singh et al., 2018). Furthermore, the inoculated species demonstrated significantly better responses in terms of maize plant height.

3.2.1 Plant fresh biomass (g pot^{-1})

Among P strains, the highest fresh shoot weight of 30.17 g pot^{-1} was observed in the *Bacillus PIS7* treatment, followed closely by *Bacillus DUM7* with a fresh shoot weight of 28.69 g pot^{-1} (Table 2). In contrast, *Bacillus CUM6* displayed the lowest fresh shoot weight of 27.58 g pot^{-1}. Furthermore, among the various P levels, the highest fresh shoot weight of 32.99 g pot^{-1} was achieved with an application of 80 kg P_2O_5 ha^{-1}, followed by 60 kg P_2O_5 ha^{-1}, resulting in a fresh shoot weight of 30.13 g pot^{-1}. In comparison, the control group exhibited the smallest observation of 24.64 g pot^{-1}.

Among combined treatments, the maximum fresh shoot weight of 35.33 g pot^{-1} was observed when *Bacillus PIS7* was combined with 80 kg P_2O_5 ha^{-1}. This was closely followed by the integrated application of *Bacillus DUM7* with 80 kg P_2O_5 per hectare, resulting in a fresh shoot weight of 31.12 g pot^{-1}. Conversely, the minimum fresh shoot weight of 24.24 g pot^{-1} was recorded when only *Bacillus CUM6* was applied. These results strongly indicated that the combination of PSB strain Bacillus PIS7 and 80 kg P_2O_5 ha^{-1} significantly enhanced maize fresh shoot weight. This could be attributed to the reduced fractions of pollutants such as Cd, Pb, Cr, and Ni under contaminated conditions (Asensio et al., 2013).

3.2.2 Plant dry biomass (g pot^{-1})

Results of plant dry biomass demonstrated that among PB strains, Bacillus PIS7 achieved the highest maize dry weight of 19.40 g pot^{-1}, followed by *Bacillus DUM7* with a dry weight of 17.92 g pot^{-1} (Table 2). In contrast, *Bacillus CUM6* exhibited the lowest dry weight of 16.81 g pot^{-1}. Whereas among P levels, the highest dry shoot weight of 22.22 g pot^{-1} was achieved with an application of 80 kg P_2O_5 ha^{-1}, followed by 60 kg P_2O_5 ha^{-1}, which resulted in an observation of 19.36 g pot^{-1}. The control group displayed the lowest dry shoot weight of 13.87 g pot-1.

Furthermore, the interaction between PSB strains and different P levels exhibited that the highest dry shoot weight of 24.56 g pot^{-1} was recorded when *Bacillus PIS7* was combined with 80 kg P_2O_5 ha^{-1}. Similarly, *Bacillus DUM7* combined with 80 kg P_2O_5 ha^{-1} resulted in a dry shoot weight of 21.35 g pot^{-1}. Conversely, the lowest dry shoot weight of 13.47 g pot^{-1} was observed when *Bacillus CUM6* was applied without P supplementation.

Table 2. Effect of PSB strains and various P levels on plant height, fresh biomass, dry biomass, fresh root weight, dry root weight.

P (kg ha^{-1})	PSB Strains (cm)	Plant Height (cm)	fresh biomass (g pot^{-1})	Dry biomass (g pot^{-1})	FRW (g pot^{-1})	DRW (g pot^{-1})
0	Bacillus CUM6	91 k	24.24l	13.47l	6c	3.10 h
40	Bacillus CUM6	100 g	26.44 i	15.67i	6.9c	3.58 f
60	Bacillus CUM6	102f	28.12g	17.35g	7.7b	3.92e
80	Bacillus CUM6	93j	31.54d	20.77 d	9.5b	4.83c
0	Bacillus DUM7	106e	24.75k	13.98k	6.3c	3.30g
40	Bacillus DUM7	108d	27.55h	16.78 h	7.3b	3.77e
60	Bacillus DUM7	95i	30.34e	19.57e	8.1b	4.21d
80	Bacillus DUM7	114c	31.12b	21.35b	10a	5.14b
0	Bacillus PIS7	118b	24.64d	14.18j	6.5c	3.52f
40	Bacillus PIS7	98h	27.48c	17.69f	7.5b	3.86 e
60	Bacillus PIS7	117b	30.13b	21.17c	8.4b	4.33d
80	Bacillus PIS7	122a	32.99a	24.56a	10.5a	5.67 a
	SOV					
	PSB strain	ns	*	**	ns	*
	P	ns	**	*	ns	**
	PSB*P	ns	*	**	ns	ns
	LSD	1.69	0.04	0.02	0.17	0.007

Note: P-Phosphorus, FRW-fresh root weight, DRW- root weight, SOV-source of variation, ns-non significant.

The increase in maize plant growth and dry shoot weight can be attributed to the enhanced absorption of phosphorus (P) by the soil, leading to an accumulation of P and subsequent promotion of dry shoot weight (Wahid et al., 2016). Moreover, the present study reveals that the increased dry shoot weight of maize is not solely due to the application of 80 kg P_2O_5 ha^{-1} and *Bacillus PIS7* (PSB strain), but also the phytoremediation of pollutants, resulting in reduced pollutant accumulation. Nadeem et al. (2020) further supported the significance of rhizobacteria in developing plant resistance against the harmful effects of toxic materials such as As, Cd, Ni, and Pb.

3.2.1 Plant fresh biomass (g pot^{-1})

Among P strains, the highest fresh shoot weight of 30.17 g pot^{-1} was observed in the *Bacillus PIS7* treatment, followed closely by *Bacillus DUM7* with a fresh shoot weight of 28.69 g pot^{-1} (Table 2). In contrast, *Bacillus CUM6* displayed the lowest fresh shoot weight of 27.58 g pot^{-1}. Furthermore, among the various P levels, the highest fresh shoot weight of 32.99 g pot^{-1} was achieved with an application of 80 kg P_2O_5 ha^{-1}, followed by 60 kg P_2O_5 ha^{-1}, resulting in a fresh shoot weight of 30.13 g pot^{-1}. In comparison, the control group exhibited the smallest observation of 24.64 g pot^{-1}.

Among combined treatments, the maximum fresh shoot weight of 35.33 g pot^{-1} was observed when *Bacillus PIS7* was combined with 80 kg $P_2 O_5$ ha^{-1}. This was closely followed by the integrated application of *Bacillus DUM7* with 80 kg P_2O_5 per hectare, resulting in a fresh shoot weight of 31.12 g pot^{-1}. Conversely, the minimum fresh shoot weight of 24.24 g pot^{-1} was recorded when only *Bacillus CUM6* was applied. These results strongly indicated that the combination of PSB strain Bacillus PIS7 and 80 kg P_2O_5 ha^{-1} significantly enhanced maize fresh shoot weight. This could be attributed to the reduced fractions of pollutants such as Cd, Pb, Cr, and Ni under contaminated conditions

3.2.2 Plant dry biomass (g pot^{-1})

Results of plant dry biomass demonstrated that among PB strains, Bacillus PIS7 achieved the highest maize dry weight of 19.40 g pot^{-1}, followed by *Bacillus DUM7* with a dry weight of 17.92 g pot^{-1} (Table 2). In contrast, *Bacillus CUM6* exhibited the lowest dry weight of 16.81 g pot^{-1}. Whereas among P levels, the highest dry shoot weight of 22.22 g pot^{-1} was achieved with an application of 80 kg P_2O_5 ha^{-1}, followed by 60 kg P_2O_5 ha^{-1}, which resulted in an observation of 19.36 g pot^{-1}. The control group displayed the lowest dry shoot weight of 13.87 g pot^{-1}.

Furthermore, the interaction between PSB strains and different P levels exhibited that the highest dry shoot weight of 24.56 g pot^{-1} was recorded when *Bacillus PIS7* was combined with 80 kg P_2O_5 ha^{-1}. Similarly, *Bacillus DUM7* combined with 80 kg P_2O_5 ha^{-1} resulted in a dry shoot weight of 21.35 g pot^{-1}. Conversely, the lowest dry shoot weight of 13.47 g pot^{-1} was observed when *Bacillus CUM6* was applied without P supplementation. The increase in maize plant growth and dry shoot weight can be attributed to the enhanced absorption of phosphorus (P)

by the soil, leading to an accumulation of P and subsequent promotion of dry shoot weight (Wahid et al., 2016). Moreover, the present study reveals that the increased dry shoot weight of maize is not solely due to the application of 80 kg P_2O_5 ha $^{-1}$ and *Bacillus PIS7* (PSB strain), but also the phytoremediation of pollutants, resulting in reduced pollutant accumulation. Nadeem et al. (2020) further supported the significance of rhizobacteria in developing plant resistance against the harmful effects of toxic materials such as As, Cd, Ni, and Pb.

3.2.3 Plant root fresh weight (g pot $^{-1}$)

Maize root fresh weight was influenced by different phosphorus solubilizing bacteria (PSB) strains and P levels (Table 2). Among the PSB strains, *Bacillus PIS7* exhibited the highest root fresh weight of 8.20 g pot $^{-1}$, followed by *Bacillus DUM7* with 7.93 g pot $^{-1}$. Conversely, *Bacillus CUM6* displayed the lowest root fresh weight of 7.52 g pot $^{-1}$. Among P Levels, the highest root fresh weight of 9.97 g pot $^{-1}$ was observed with an application of 80 kg ha $^{-1}$ of P, followed by 60 kg ha $^{-1}$ of P, resulting in a root fresh weight of 8.07 g pot $^{-1}$. The control group exhibited the lowest root fresh weight of 6.27 g pot $^{-1}$. The interaction between PSB strains and various P levels did not significantly impact maize root fresh weight.

The efficacy of PSB in making phosphorus available for plant nourishment has been a subject of ongoing debate (Raymond et al., 2021). Nevertheless, evidence suggests that abiotic stresses, such as nutritional, salinity, or drought stress, can significantly influence the capacity of PSB strains to support plant growth by creating a favorable soil environment that promotes increased root fresh weight (Mendoza-Labrador et al., 2021).

3.2.4 Plant root dry weight (g pot $^{-1}$)

Among PSB strains, *Bacillus PIS7* exhibited the maximum root dry weight of 4.35 g pot $^{-1}$, followed by Bacillus DUM7, with a dry weight of 4.11 g pot $^{-1}$ (Table 2). In contrast, *Bacillus CUM6* displayed the lowest root dry weight of 3.86 g pot $^{-1}$. Additionally, different levels of phosphorus application demonstrated a significant improvement in root dry weight. The highest root dry weight of 5.21 g pot $^{-1}$ was achieved with an application of 80 kg ha $^{-1}$ of P, while the closest observation was obtained with 60 kg ha $^{-1}$ of P_2O_5, resulting in a root dry weight of 4.15 g pot $^{-1}$. Conversely, the smallest finding of 3.31 g pot $^{-1}$ was recorded in the no-dosage treatment.

The interaction between PSB strains and various P doses notably influenced maize root dry weight. The highest root dry weight of 5.67 g pot $^{-1}$ was observed when *Bacillus PIS7* was combined with 80 kg ha $^{-1}$ of P_2O_5. Similarly, the integrated application of *Bacillus DUM7* with 80 kg ha $^{-1}$ of P_2O_5 resulted in a root dry weight of 5.14 g pot $^{-1}$. On the other hand, the lowest maize root dry weight of 3.10 g pot $^{-1}$ was recorded with the sole application of *Bacillus CUM6*. These results strongly suggest that PSB strains *Bacillus PIS7* and 80 kg ha $^{-1}$ of P_2O_5 significantly increased maize root dry weight. This effect may be attributed to the reduction of harmful substances such as Cd, Pb, Cr, and Ni in contaminated conditions.

The improvement in maize plant dry root

weight can be attributed to the reduction of bioavailable fractions of toxic metals in the soil resulting from the combined effects of PSB strains and P fertilization. Furthermore, it has been a subject of ongoing debate whether PSB truly facilitates phosphorus availability for plant nourishment (Raymond et al., 2021). Multiple lines of evidence suggest that abiotic stresses, such as nutritional, salinity, or drought stress, significantly influence the capacity of these bacteria to support plant growth by creating a favorable soil environment, which contributes to an increase in plant root dry weight (Mendoza-Labrador et al., 2021). The findings by Pardo-Díaz et al. (2021) support the significant enhancement of plant dry root weight through the combination of PSB strains with synthetic doses. Moreno-Galván et al. (2020) also observed the efficient improvement of plant vegetative growth through the use of various PSB strains.

3.3 Effect of rhizobacteria and applied phosphorous concerning the focus of phosphorous and heavy metals in soil

3.3.1 Soil AB-DTPA extractable P (mg kg $^{-1}$)

Application of microbial strains can increase the uptake of macro- and micronutrients, thereby enhancing plant growth. The use of various PSB strains positively enhanced AB-DTPA extractable P, thus the maximum AB-DTPA extractable P of 5.14 and 5.14 mg kg^{-1} was noticed in *Bacillus PIS7* and *Bacillus DUM7* treatment pots whereas the minimum AB-DTPA extractable P of 5.03 mg kg^{-1} was noted in *Bacillus CUM6* (Figure 1A). Among different P rates, the highest AB-DTPA extractable P of 7.07 mg kg^{-1} was resulted in 80 kg ha^{-1} P_2O_5 proceeded by 60 kg ha^{-1} which attained the observations of 5.74 mg kg^{-1}, after then the least observations of P 3.17 mg kg^{-1} was deduced in control. The combined potential of PSB strains and different P levels on AB-DTPA extractable P was found statistically efficient. The higher observations of P 7.13 mg kg^{-1} were found where *Bacillus PIS7* along with 80 kg ha^{-1} P_2O_5 applied, which was closely followed by the integrated application of *Bacillus CUM6* and 80 kg ha^{-1} P_2O_5 that attained the observations of 7.06 mg kg^{-1}, whereas the minimum AB-DTPA extractable P of 3.11 mg kg^{-1} was recorded where only *Bacillus CUM6* applied. The present results indicated that as the levels of P increased the AB-DTPA extractable P linearly increases with all PSB strains but the more effective strains was *Bacillus PIS7* and Bacillus DUM which shows statistically non-significant differences and at effect in increasing the availability of P in soil. Moreover, these PSB strains also played a significant role in reducing the heavy metal content in contaminated environments (Teng et al., 2019).

Macronutrients and microbes enhanced the availability of soil phosphorous (Fahsi et al., 2021; Wang et al., 2009; Sarwar et al., 2010). Enhanced P uptake in plants increases the production of glutathione, which protects against Cd-induced membrane damage (Wang et al., 2009).

3.3.2 Soil AB-DTPA extractable Cd (mg kg^{-1}).

The soil Cd content was significantly stabilized with PSB strains (Figure 1). Among different PSB stains, the maximum soil Cd content of 5.49 mg kg^{-1} was deduced in *Bacillus CUM6* strain after then *Bacillus*

DUM7, resulted soil Cd of 5.46 mg kg^{-1}, although the lesser soil Cd fractions of 5.44 mg kg^{-1} was deduced in *Bacillus PIS7*. Making advantage of phosphorous doses significantly reduced Cd fractions in soil. Among various P levels, the maximum soil Cd content 8.16 mg kg^{-1} was seduced in no-supplemented site after then P application 40 kg ha^{-1} attained soil Cd concentration of 6.38 mg kg^{-1}, and the least observations of 2.92 mg kg^{-1} was noticed in 80 kg P_2O_5 ha^{-1} treatment pot. The interaction among PSB strains and various P levels over soil Cd was found statistically significant. Among the interaction data, the maximum Cd content in soil of 8.21 mg kg^{-1} was found where only *Bacillus CUM6* and was closely followed by *Bacillus DUM7* without P application deduced the observations of 8.16 mg kg^{-1}, and the minimum findings (2.88 mg kg^{-1}) was noted in *Bacillus PIS7* with 80 kg P_2O_5 ha^{-1} supplementation.

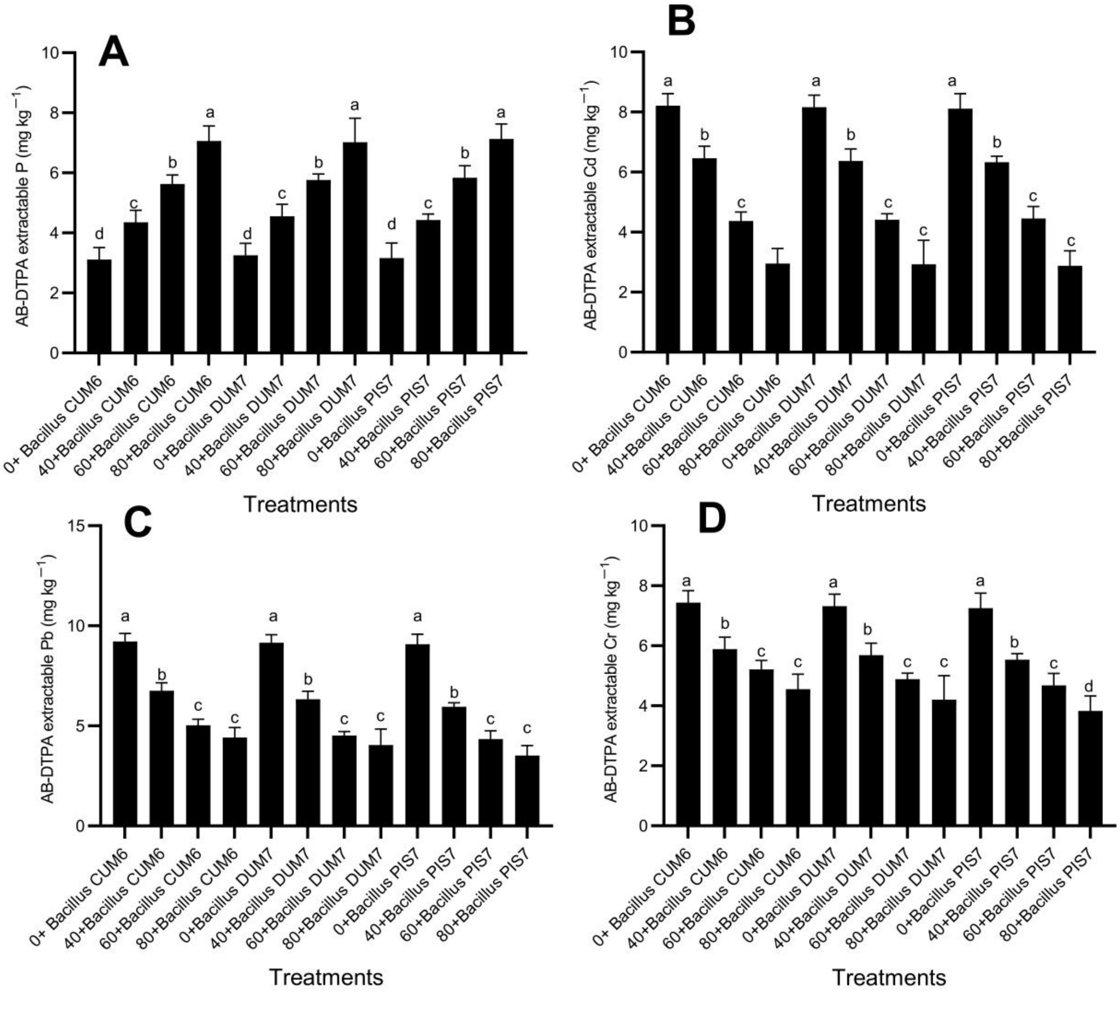

Figure 1. Effect of PSB strains and various P levels on soil (A) B-DTPA extractable P (mg kg-1), (B) AB-DTPA extractable Cd (mg kg^{-1}), (C) AB-DTPA extractable Pb (mg kg^{-1}) and (D) AB-DTPA extractable Cr (mg kg^{-1}).

Findings further showed that among the heavy metals Cd content drastically and linearly reduced with applied phosphorous and PSB strains, indicated antagonism between P added occurred during plant growth. Phosphorous may immobilized or adsorbed the Cd in soil. P fertilization efficiently reduced the bioavailable fraction of Cd inside soil (Falamaki et al. 2016; Huang et al., 2016; Lu et al., 2017). Although lowering the bioavailability of Cd and immobilizing it in polluted soils may be accomplished with P-based amendments (Liang et al., 2014 The process of triple super phosphate remediation most likely caused metal phosphate mineral to precipitate or co-precipitate, which resulted in P-induced metal immobilization (Hashimoto et al., 2009).

3.3.3 Soil AB-DTPA extractable Pb (mg kg^{-1})

The use of PSB strains in combination with different P levels significantly decreased the concentration of AB-DTPA extractable Pb content (Figure 1). The use of various PSB strains positively reduced AB-DTPA extractable Pb content, thus higher Pb content of 6.36 mg kg^{-1} was noticed in *Bacillus CUM6* whereas the lesser observations of 5.73 mg kg^{-1} were noted in *Bacillus PIS7*. The present results also depicted that different P application rates significantly decreased the AB-DTPA extractable Pb content. Among different P levels, the maximum soil Pb of 9.15 mg kg^{-1} was resulted in control although 60 kg P_2O_5 ha^{-1} which attained AB-DTPA extractable Pb content of 6.35 mg kg^{-1} while the minimum AB-DTPA extractable Pb content of 4.00 mg kg^{-1} was recorded in 80 kg P_2O_5 ha^{-1} treatment. The interaction among PSB strains and several P doses on AB-DTPA extractable Pb content was located significantly. The maximum observations of 9.22 mg kg^{-1} was found in *Bacillus CUM6* with no P application treatments that was followed by the treatment where only *Bacillus DUM7* was applied that resulted in AB-DTPA extractable Pb of 9.15 mg kg^{-1} whereas the least Pb content of 3.52 mg kg^{-1} was recorded in *Bacillus PIS7* along with 80 kg ha^{-1} P_2O_5 application.

Previous results were similar to the present findings in which various bacterial species stabilized the concentrations of Pb under soil due to their high surface area and bio-sorbent capability (Sharma and Archana, 2016; Hussain et al., 2023). The phosphate solubilizing bacteria releases phosphates inside the soil that makes phosphate mineralizing precipitates with toxic metals that significantly reduce their content in the soil (Xiao et al., 2021; Priya et al., 2022

3.3.4 Soil AB-DTPA extractable Cr (mg kg^{-1})

The results showed that PSB strains efficiently decreased the AB-DTPA extractable Cr, thus the maximum observations of 5.77 mg kg^{-1} was noticed in *Bacillus CUM6* while the minimum soil Cr of 5.32 mg kg^{-1} was located in *Bacillus PIS7* strain (Figure 1). The AB-DTPA extractable Cr was significantly reduced with various P levels. The maximum soil Cr of 7.34 mg kg^{-1} was located in control that was followed by 40 kg P_2O_5 ha^{-1} treatment resulted soil Cr of 5.71 mg kg^{-1} while the minimum soil Cr of 4.19 mg kg^{-1} was deduced in 80 kg P_2O_5 ha^{-1} treatment. The combined effect of PSB

strains and different P levels on plant AB-DTPA extractable Cr was noted as effective. The maximum AB-DTPA extractable Cr content of 7.44 mg kg^{-1} was found where *Bacillus CUM6* with no P application treatment that was followed by the treatment where only *Bacillus DUM7* resulted in 7.32 mg kg $^{-1}$ soil Cr whereas the minimum observations of 3.83 mg kg $^{-1}$ was located in *Bacillus PIS7* along with 80 kg ha $^{-1}$ P_2O_5 supplementation.

Through the beneficiary activities of soil biota along with other chemical mechanisms, the PSB efficiently lowered the bioavailable fractions of pollutants inside beneath the soil, preventing crops from absorbing those (Teng et al., 2019). Functional bacteria are thought to chelate heavy metals (Chen et al., 2019). Metallothionein, iron transporters, and elements of the bacterial pore are also thought to reduce the concentrations of Cd and Pb in the soil or solution (El-Meihy et al., 2019). Functional bacteria are thought to chelate heavy metals through several chemical mechanisms. Metallothionein, iron transporters, and elements of the bacterial pore are also thought to reduce the concentrations of Cd and Pb in the soil or solution (Chen et al., 2019; El-Meihy et al., 2019).

3.3.5 Soil AB-DTPA extractable Ni (mg kg^{-1})

The AB-DTPA extractable Ni was significantly decreased with PSB strains (Figure 2A). Among different PSB stains, the highest observations of 4.80 mg kg $^{-1}$ was deduced in *Bacillus CUM6* strain and the lesser observations of 4.20 mg kg $^{-1}$ was located in *Bacillus PIS7*. The use of synthetic fertilizers as phosphorous significantly reduced AB-DTPA extractable Ni. Among various P levels, the maximum AB-DTPA extractable Ni of 6.04 mg kg^{-1} was seduced in no-supplemented site although the treatment where P was applied at 40 kg P_2O_5 ha^{-1} that attained AB-DTPA extractable Ni of 4.62 mg kg $^{-1}$ and the least observations of 3.26 mg kg $^{-1}$ was noticed in 80 kg ha $^{-1}$ P_2O_5 treatment. The combination among PSB strains and various P levels on AB-DTPA extractable Ni was found significant. Among the interaction data, the optimal findings of 6.11 mg kg $^{-1}$ was found in *Bacillus CUM6* with no phosphorous application was closely followed by *Bacillus DUM7* with no P fertilizer treatment that resulted in 6.04 mg kg $^{-1}$ soil Ni whereas the lesser outcomes of 2.88 mg kg $^{-1}$ was noted in *Bacillus PIS7* with 80 kg P_2O_5 ha^{-1} application.

Ni in soil was significantly reduced with the use of bacterial strains along with other fertilizers as inspected by (Gordegir et al., 2019). The pollutants content in an artificially environment contaminated was positively decreased with various phosphate solubilizing bacterial strains as deduced by (Janeena et al., 2023). Zhang et al. (2021) inspected those different bacterial strains efficiently reduced the harmful gradients inside the soil. The content of toxic metals in artificially contaminated soil were efficiently stabilized with bacterial strains as seduced by (Mehrotra et al., 2021; Wang et al., 2022).

3.4 Effect of rhizobacteria and applied phosphorous on the important properties of soil

3.4.1 Soil organic matter (%)

The application of PSB strains significantly

improved the content of soil organic matter (Wahid et al., 2016; Li and Gao., 2019; Rizwan et al., 2016). In the current study PSB strains efficiently increased the soil organic matter, thus the maximum soil organic matter (0.67 and 0.66 %) was noticed in *Bacillus PIS7* and *Bacillus DUM7* strains while the minimum soil organic matter (0.65 %) was recorded in *Bacillus CUM6* (Figure 2B). The soil organic matter was significantly improved with various P levels. The maximum soil organic matter (0.72 %) was located in 80 kg ha $^{-1}$ P_2O_5 although 60 kg P_2O_5 ha^{-1} produced soil organic matter (0.68 %) and the lesser observations (0.60 %) was noticed in no-supplemented site. The interactive effective of both PSB strains and different P doses SOM was noted effective. .

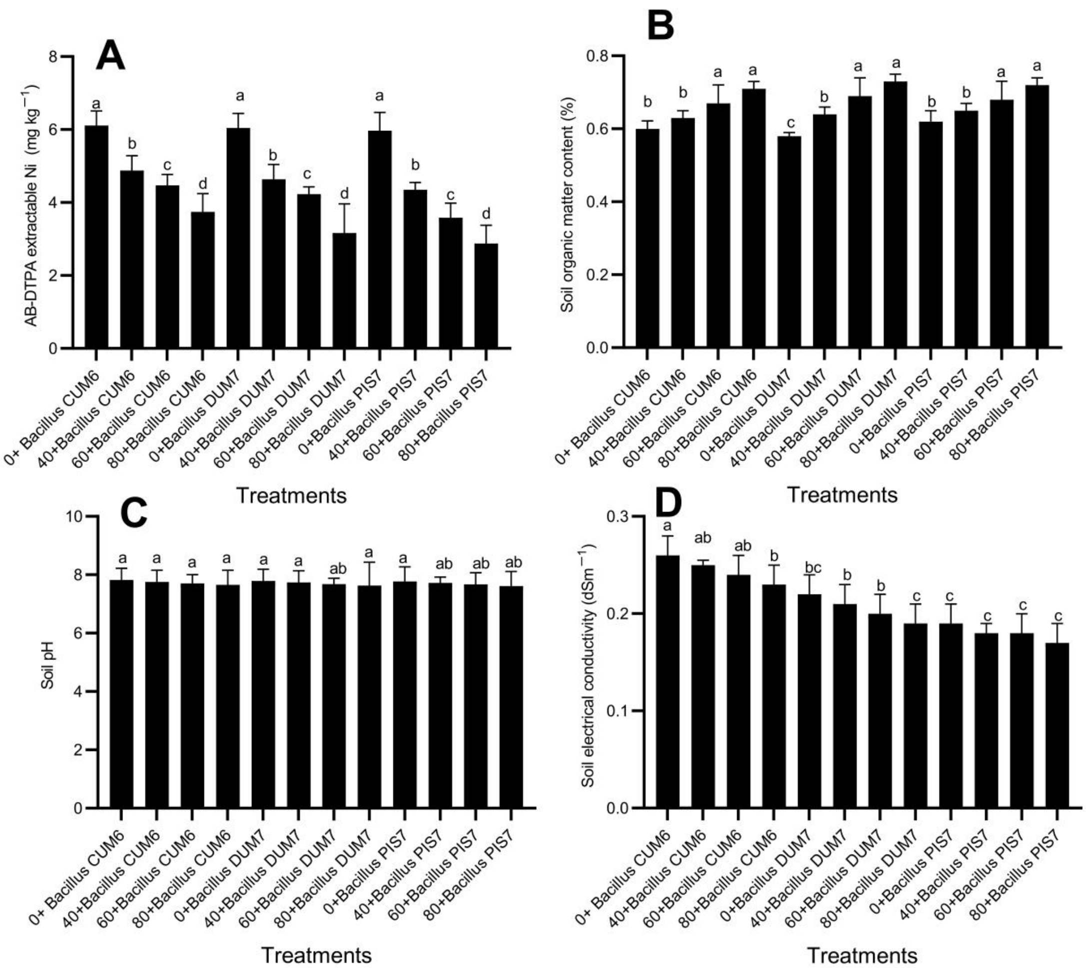

Figure 2. Effect of PSB strains and various P levels on soil (A) extractable Ni (B) soil organic matter (%) (C) soil pH and (D) soil electrical conductivity (dSm^{-1})

The maximum soil organic matter (0.73 %) was recorded where *Bacillus DUM7* and 80 kg P_2O_5 ha^{-1} was incorporated after then *Bacillus PIS7* along with 80 kg P_2O_5 ha^{-1} which resulted soil organic matter of 0.72 % whereas the minimum soil organic matter (0.58 %) was noted where *Bacillus DUM7* without P level was applied. The results showed that all PSB strains increased soil organic matter content linearly as P levels rose, however *Bacillus PIS7* and Bacillus. DUM were the most efficient strains, showing statistically non-significant differences and having an impact on increasing the content of organic matter in soil. These PSB strains also significantly contributed to lowering the level of heavy metals in the contaminated surroundings

3.4.2 Soil pH

The application of PSB strains significantly decreased the pH of the soil (Wahid et al., 2016; Li and Gao., 2019; Rizwan et al., 2016). The soil pH was significantly decreased with PSB strains. Among different PSB stains, the maximum soil pH of 7.73 was deduced in *Bacillus CUM6* after then *Bacillus DUM7*, resulted 7.71 findings whereas the least observations of 7.69 was noted in *Bacillus PIS7* treatment pot. The use of synthetic fertilizers as phosphorous significantly reduced soil pH. Among various P levels, the maximum soil pH of 7.79 was noted in no P amended pots after then P dose 40 kg ha^{-1} P_2O_5 that attained the outcomes of 7.74, and the lesser outcomes of 7.63 was noticed in where 80 kg P_2O_5 ha^{-1} treatment pot. The interaction among PSB strains and various P levels over soil pH was found non-significant (Figure 2C).

3.4.3 Soil electrical conductivity (dSm^{-1})

Figure 2D illustrated the mean results of soil electrical conductivity as affected by PSB strains and different P levels. The results showed that PSB strains efficiently decreased the soil electrical conductivity, thus the maximum soil electrical conductivity of 0.24 dSm^{-1} was noticed in *Bacillus CUM6* followed by *Bacillus PIS7* attained the soil electrical conductivity of 0.20 dSm^{-1}, while the minimum soil electrical conductivity of 0.18 dSm^{-1} was recorded in *Bacillus PIS7*. Among the various P levels, the maximum soil electrical conductivity of 0.22 dSm^{-1} was noted in 0 kg ha^{-1} P_2O_5 although 40 and 60 kg ha^{-1} P_2O_5 resulted in soil electrical conductivity of 0.21 and 0.21 dSm^{-1}, while the minimum soil electrical conductivity of 0.20 dSm^{-1} was noticed in 80 kg P_2O_5 ha^{-1} treated pot. The interactive effective of both PSB strains and different P levels on soil electrical conductivity was found statistically non-significant. Chemical fertilizer considerably increased soil EC (Shazma et al., 2016; Bhatt et al., 2018; Pal et al., 2007). On other hand *Bacillus PIS7* results to decrease EC.

3.5 Effect of rhizobacteria and applied phosphorous on the concentration of phosphorous and heavy metals in plant

3.5.1 Plant P (%)

Application of microbial strains enhance the phosphorus accumulation in plants (Wang et al., 2022; Sarwar et al., 2010). In this study we also observed that PSB strains positively enhanced plant P concentration, thus the maximum plant P fractions of 0.19 % was noticed in *Bacillus PIS7* treatment pot

followed by *Bacillus DUM7* treatment pot which attained plant P concentration of 0.16 % whereas the minimum fractions of P 0.14 % was located in *Bacillus CUM6* (Table 3). The present results also depicted that different P application rates significantly improved plant P concentration. Among different P levels, the maximum plant P concentration of 0.23 % resulted in 80 kg ha^{-1} P_2O_5 although 60 kg ha^{-1} P_2O_5 attained maize plant P concentration of 0.19 %, although the lesser observations of 0.08 % was located in control. The combination of PSB strains and several P doses on P fractions was allocated as statistically significant. The higher observations of 0.26 % was found where *Bacillus PIS7* along with 80 kg P_2O_5 ha^{-1} applied, which was closely followed by the integrated application of *Bacillus DUM7* and 80 kg ha^{-1} P_2O_5 resulted in the findings of 0.23 % although the least findings of 0.07 % was seduced where only *Bacillus CUM6* applied. The overall results indicated that as the applied P levels in soil increases the accumulation of P in plant shoot correspondingly increased and showed significant variation. On the other hand, the effect of PSB strains also increases the P concentration in plant shoot in a linear fashion; however, the effect of *Bacillus PIS7* was more pronounced as in comparison to PSB strains such as *Bacillus DUM7* and *Bacillus CUM6* in increasing the concentration of P.

3.5.2 Plant Shoot Cd (mg kg^{-1})

The use of various PSB strains positively minimized the content of Cd in maize shoot, thus the maximum shoot Cd content of 6.17 mg kg^{-1} was noticed in *Bacillus CUM6* treatment followed by *Bacillus DUM7* treatment that attained shoot Cd of 6.05 mg kg^{-1} whereas the minimum Cd level in maize shoot of 6 mg kg^{-1} was noted in *Bacillus PIS7* (Table 3). The present results also depicted that different P application rates significantly decreased Cd concentration in shoot. Among different P levels, the maximum shoot Cd of 8.34 mg kg^{-1} was resulted in control that was followed by 40 kg P_2O_5 ha^{-1} which attained shoot Cd content of 7.18 mg kg^{-1} although the lesser shoot Cd fractions of 3.37 mg kg^{-1} was located in 80 kg ha^{-1} P_2O_5. The combination among PSB strains and different P levels on shoot Cd content was found significant. The maximum shoot Cd content of 8.45 mg kg^{-1} was found where *Bacillus CUM6* without P application that was closely followed by the sole application of *Bacillus DUM7* that attained the Cd shoot concentration of 8.33 mg kg^{-1} and the lowered shoot Cd of 3.31 mg kg^{-1} was recorded where *Bacillus DUM7* and 80 kg P_2O_5 ha^{-1} was applied. Phosphatic fertilizers efficiently lowered the content of soil Cd that ultimately inhibits the translocation of Cd from soil to plant (Huang et al., 2016; Cao et al., 2009; Zhu et al., 2015).

3.5.2 Plant Shoot Pb (mg kg^{-1})

The use of various PSB strains positively reduced Shoot Pb content, thus the maximum Shoot Pb content of 5.64 mg kg^{-1} was noticed in *Bacillus CUM6* treatment pot whereas the minimum Shoot Pb content of 4.93 mg kg^{-1} was noted in *Bacillus PIS7* (Table 3). The present results also depicted that different P application rates significantly decreased the Shoot Pb content.

Among different P levels, the maximum Shoot Pb content of 8.44 mg kg^{-1} was

resulted in control although 60 kg P_2O_5 ha^{-1} which attained Shoot Pb content of 5.60 mg kg^{-1} and the lessened shoot Pb 3.21 mg kg^{-1}

Table 3. Effect of PSB strains and various P levels on P(%) in leaves and Cd, Pb, Cr, Ni in both shoot and roots of maize plant

P Levels	PSB Strains	P(%)	Shoot (mg kg $^{-1}$)				Root (mg kg $^{-1}$)			
			Cd	Pb	Cr	Ni	Cd	PB	Cr	Ni
0	Bacillus CUM6	0.07 h	8.45 a	8.77 a	3.22 a	4.12 a	9.77 b	9.65 a	8.35 a	7.66 a
40	Bacillus CUM6	0.12 f	7.25 d	6.02 d	2.56 d	3.04 d	8.92 c	7.11 d	7.42 c	6.43 d
60	Bacillus CUM6	0.16 e	5.56 g	4.21 g	1.87 g	2.45 f	7.23 g	5.25 g	6.77 f	6.02 f
80	Bacillus CUM6	0.21 c	3.34 j	3.55 j	1.01 j	1.88 i	5.67 i	4.64 j	6.10 i	5.29 i
0	Bacillus DUM7	0.08 gh	8.33 b	8.44 b	3.13 b	4.07 b	9.81 a	9.46 b	8.31 a	7.59 b
40	Bacillus DUM7	0.15 e	7.11 f	5.76 e	2.21 e	2.88 e	8.87 d	6.85 e	7.23 d	6.19 e
60	Bacillus DUM7	0.19 d	5.44 h	3.97 h	1.45 h	2.21 h	7.45 f	4.97 h	6.42 g	5.78 h
80	Bacillus DUM7	0.23 b	3.31 l	3.12 k	0.72 k	1.55 j	5.45 j	4.18 k	5.78 j	4.71 k
0	Bacillus PIS7	0.09 g	8.24 c	8.11 c	3.04 c	4.01 c	9.78 ab	9.23 c	8.23 b	7.52 c
40	Bacillus PIS7	0.19 d	7.17 e	5.02 f	2.04 f	2.33 g	8.82 e	6.15 f	7.08 e	5.87 g
60	Bacillus PIS7	0.22 bc	5.23 i	3.62 i	1.17 i	1.89 i	7.16 h	4.76 i	6.21 h	5.13 j
80	Bacillus PIS7	0.26 a	3.37 k	2.96 l	0.42 l	1.33 k	5.21 k	3.98 l	5.36 k	4.43 l
	SOV									
	PSB strain	*	*	*	**	*	*	**	*	*
	P	ns	*	*	**	**	*	**	*	*
	PSB*P	**	*	*	**	*	*	*	ns	**

Note: . Values in the column followed by the same letters are statistically the same. SOV-Source of variation, P-Phosporous, ns-non significant, * - significant at 5 % ($P < 0.05$). **- Significant at 1 % ($P < 0.01$).

was allocated in 80 kg ha $^{-1}$ P_2O_5 treatment. The combination among PSB strains and different P levels on Shoot Pb content was found significant. The maximum Shoot Pb content of 8.77 mg kg^{-1} was found where *Bacillus CUM6* with no P application treatments that was followed by the treatment where only *Bacillus DUM7* was applied that resulted in AB-DTPA extractable Pb of 8.44 mg/kg whereas the minimum Shoot Pb content of 2.96 mg kg $^{-1}$ was recorded in *Bacillus PIS7* along with 80 kg ha $^{-1}$ P_2O_5 supplementation. Application of bacterial strains inhibits the transformation of soil pollutants to plants (Quezada-Hinojosa et al., 2015; Volpe et al., 2015; Sharma and Archana., 2016; Chen et al., 2019)

3.5.3 Plant Shoot Cr (mg kg $^{-1}$)

The results showed that PSB strains efficiently decreased the Shoot Cr content, thus the maximum Shoot Cr content of 2.16 mg kg $^{-1}$ was noticed in *Bacillus CUM6* while the minimum Shoot Cr content of 1.67 mg kg^{-1} was recorded in the *Bacillus PIS7* strain. The Shoot Cr content was significantly reduced with various P levels (Table 3). The maximum shoot Cr 3.13 mg kg $^{-1}$ was allocated in no-dosage treatment although 40 kg P_2O_5 ha^{-1} treatment resulted in a shoot Cr content of 2.27 mg kg $^{-1}$ and the lessened shoot Cr content of 0.72 mg/kg was noticed in 80 kg ha $^{-1}$ P_2O_5 treatment. The interactive effectiveness of both PSB strains and different P levels on plant Shoot Cr content was noted as effective. The maximum Shoot Cr content of 3.22 mg kg^{-1} was found where *Bacillus CUM6* with no P application treatment that was followed by the treatment where only *Bacillus DUM7* resulted in Shoot Cr content of 3.13 mg/kg although the lessened shoot Cr content of 0.42 mg kg^{-1} was recorded in *Bacillus PIS7* along with 80 kg P_2O_5 ha^{-1} application.

3.5.4 Plant Shoot Ni (mg kg $^{-1}$)

The bacterial strains release various acid in soil that makes precipitates with pollutants and reduced the accumulation of metals in plants (Zhao et al., 2022).The Shoot Ni content was significantly decreased with PSB strains. Among different PSB stains, the maximum Shoot Ni content of 2.87 mg kg^{-1} was deduced in the *Bacillus CUM6* strain whereas the minimum Shoot Ni content of 2.39 mg kg $^{-1}$ was located in *Bacillus PIS7* (Table 3). It was also suspected that phosphorous efficiently reduced shoot Ni content. Among various P levels, the maximum Shoot Ni content of 4.07 mg kg^{-1} was located in a no-supplemented site although P at 40 kg P_2O_5 ha $^{-1}$ attained Shoot Ni content of 2.75 mg kg $^{-1}$ although the lessened shoot Ni 1.59 mg kg $^{-1}$ was noticed in 80 kg P ha^{-1}. The interaction among PSB strains and various P levels on Shoot Ni content was found significant. Among the interaction data, the maximum Shoot Ni content of 4.12 mg kg $^{-1}$ was found in *Bacillus CUM6* with no phosphorous application that was closely followed by *Bacillus DUM7* with no P fertilizer treatment that resulted in Shoot Ni content 4.07 mg/kg although the lessened Shoot Ni content of 1.33 mg/kg was located in *Bacillus PIS7* with 80 kg ha $^{-1}$ P_2O_5 supplementation. Bacterial strains reduced the level of metals in plants as it stabilized the fractions of metals and inhibits their uptake (Liu et al., 2020; Chen et al., 2019: Zeng et al., 2017)

3.5.5 Plant Root Cd (mg kg^{-1})

P fertilization efficiently reduced the bioavailable fractions of Cd inside the soil which inhibits their entrance to plant roots (Falamaki et al., 2016). The observations revealed that PSB strains efficiently lessened root Cd, thus the maximum root Cd of 7.90 and 7.89 mg kg^{-1} was noticed in *Bacillus CUM6* and *Bacillus DUM7* while the minimum root Cd of 7.74 mg kg^{-1} was recorded in *Bacillus PIS7* (Table 3). The maize root Cd was significantly reduced with various P levels. The maximum root Cd of 9.79 mg/kg was allocated in control although 40 kg ha^{-1} P_2O_5 resulted in root Cd of 8.87 mg/kg while the lesser root Cd of 5.44 mg/kg was noticed in 80 kg ha^{-1} P_2O_5. The interactive effect of both PSB strains and different P levels on root Cd was noted as effective. The maximum root Cd of 9.77 mg kg^{-1} was recorded where only *Bacillus PIS7* was incorporated after the sole application of *Bacillus DUM7* resulted in root Cd of 9.81 mg/kg although the lessened root Cd of 5.21 mg/kg was deduced where *Bacillus PIS7* with 80 kg P_2O_5 ha^{-1} was applied.

The process of TSP remediation most likely caused metal phosphate minerals to precipitate or coprecipitate, which resulted in P-induced metal immobilization (Hashimoto et al., 2009). The P dosages efficiently lessened the bioavailable fractions of soil Cd thus decreasing its storage in plant roots as inspected by Cao et al. (2009). Zhu et al. (2015) suspected that the application of P fertilizers positively reduced soil Cd which inhibits their transfer from soil to plant. Although lowering the bioavailability of Cd and immobilizing it in polluted soils may be accomplished with P-based amendments which helps in the reduction of Cd accumulation in plant roots (Liang et al., 2014).

3.5.6 Plant Root Pb (mg kg^{-1})

Microorganisms are crucial to the chemical transformation of heavy metals and can both mobilize and immobilize them (Gadd, 2004). The use of various PSB strains positively reduced root Pb content, thus the maximum root Pb content of 6.66 mg kg^{-1} was noticed in *Bacillus CUM6* treatment whereas the minimum root Pb content of 6.03 mg kg^{-1} was noted in *Bacillus PIS7* (Table 3). The present results also depicted that different P application rates significantly decreased the root Pb content. Among different P levels, the maximum root Pb content of 9.45 mg/kg was located in control although 60 kg P_2O_5 ha^{-1} which attained root Pb content of 6.70 mg/kg although the lessened root Pb 4.27 mg kg^{-1} was observed in 80 kg ha^{-1} P_2O_5 treatment. The combination among PSB strains and different P levels on root Pb content was found significant. The maximum root Pb content of 9.65 mg kg^{-1} was found where *Bacillus CUM6* with no P application treatments that was followed by the treatment where only *Bacillus DUM7* was applied that resulted in root Pb of 9.46 mg/kg although the lesser root Pb content of 3.98 mg/kg was recorded in *Bacillus PIS7* along with 80 kg P_2O_5 ha^{-1} application. Different rhizosphere bacteria immobilize metals by excreting organic compounds and viscous slime outside the bacterial cell for a variety of purposes, including adherence to surfaces, protection, and water retention (Madhaiyan et al., 2011; El Sayed et al., 2015).

Table 4. Effect of PSB strains and various P levels on plant uptake of P, Cd, Pb, Cr, Ni.

P Levels	PSB Strains	Plant uptake (mg pot^{-1}).				
		P	Cd	Pb	Cr	Ni
0	Bacillus CUM6	0.011 j	5.69 d	5.91 a	2.17 ab	2.77 b
40	Bacillus CUM6	0.026 i	5.68 c	4.72 d	2.01 c	2.38 d
60	Bacillus CUM6	0.050 f	4.82 g	3.65 i	1.62 f	2.13 f
80	Bacillus CUM6	0.084 c	3.56 i	3.69 h	1.05 i	1.95 i
0	Bacillus DUM7	0.013 j	5.82 c	5.90 a	2.19 a	2.84 a
40	Bacillus DUM7	0.033 h	5.97 b	4.83 c	1.85 d	2.42 c
60	Bacillus DUM7	0.060 e	5.32 f	3.88 f	1.42 g	2.16 e
80	Bacillus DUM7	0.093 b	3.53 i	3.33 j	0.77 j	1.65 j
0	Bacillus PIS7	0.015 j	5.84 c	5.75 b	2.16 c	2.84 a
40	Bacillus PIS7	0.040 g	6.34 a	4.44 e	1.80 e	2.06 g
60	Bacillus PIS7	0.070 d	5.54 e	3.83 g	1.24 h	2.00 h
80	Bacillus PIS7	0.106 a	4.14 h	3.63 i	0.52 k	1.63 j
SOV						
PSB strain		*	ns	*	**	*
P		**	*	*	*	**
PSB*P		**	**	*	**	**

Note: Values in the column followed by the same letters are statistically the same. SOV-Source of variation, P-Phosphorous, ns-non significant, * - significant at 5 % ($P < 0.05$). **- Significant at 1 % ($P < 0.01$).

3.5.7 Plant Root Cr (mg kg^{-1})

Bacterial strains reduced the level of metals in plant parts (Mehrotra et al., 2021: Wang et al., 2022: Zhang et al., 2021). The results showed that PSB strains efficiently decreased the root Cr, thus the maximum root Cr of 7.16 mg kg^{-1} was noticed in *Bacillus CUM6* while the minimum root Cr content of 6.72 mg kg^{-1} was recorded in *Bacillus PIS7* strain. The root Cr was significantly reduced with various P levels (Table 3). The maximum root Cr content of 8.30 mg/kg was allocated in control although 40 kg P_2O_5 ha^{-1}

treatment resulted root Cr of 7.24 mg/kg although the lessened root Cr of 5.75 mg/kg was noticed in 80 kg ha^{-1} P_2O_5 treatment. The interactive effective of both PSB strains and different P levels on plant root Cr was

noted effective. The maximum root Cr content of 8.35 mg kg^{-1} was found where *Bacillus CUM6* with no P application treatment that was followed by the treatment where only *Bacillus DUM7* that resulted root Cr content of 8.31 mg/kg although the least root Cr content of 5.36 mg/kg was recorded in *Bacillus PIS7* along with 80 kg P_2O_5 ha^{-1} application.

3.5.7 Plant Root Ni (mg kg^{-1})

Microorganisms are crucial to the chemical transformation of heavy metals and can both mobilize and immobilize them (Gadd, 2004; Wang et al., 2022). The root Ni was significantly decreased with PSB strains. Among different PSB stains, the maximum root Ni of 6.35 mg kg^{-1} was deduced in *Bacillus CUM6* strain whereas the minimum root Ni of 5.74 mg kg^{-1} was allocated in *Bacillus PIS7* (Table 3). Phosphorous doses efficiently reduced root Ni. Among various P levels, the maximum root Ni of 7.59 mg kg^{-1} was observed in control although P at the rate of 40 kg P_2O_5 ha^{-1} attained root Ni of 6.16 mg kg^{-1} while the minimum root Ni of 4.81 mg kg^{-1} was noticed in 80 kg ha^{-1} P_2O_5 treatment. The combination of PSB strains and various P levels on root Ni was found significant. Among the interaction data, the maximum root Ni of 7.66 mg kg^{-1} was found in *Bacillus CUM6* with no phosphorous application that was closely followed by *Bacillus DUM7* with no P fertilizer treatment that resulted in root Ni content of 7.59 mg/kg although the lessened observations of 4.43 mg/kg was located in *Bacillus PIS7* with 80 kg ha^{-1} P_2O_5 application.

Reduced availability of heavy metals was associated with how distinct inorganic phosphorus forms were affected by phosphorus-dissolving bacteria (Liu et al., 2021). It has been demonstrated that bacteria can change heavy metals charge and surface-bound precipitates (Mehrotra et al., 2021).

3.6 Effect of rhizobacteria and applied phosphorous on the uptake of phosphorous and heavy metals by plants

3.6.1 Plant P uptake (mg pot^{-1})

Application of phosphorus positively boosted soil P fractions and balances the negative effects of Cd and increases crop output that helps their uptake in plants (Sarwar et al., 2010). Enhancing P uptake in plants increases the production of glutathione, which protects against Cd-induced membrane damage (Wang et al., 2009). The results showed that PSB strains efficiently increased the plant P total uptake, thus the maximum P uptake of 0.058 mg pot^{-1} was noticed in *Bacillus PIS7* followed by *Bacillus DUM7* attained the P uptake of 0.050 mg pot^{-1}, while the minimum P uptake of 0.043 mg pot^{-1} was recorded in *Bacillus CUM6* (Table 4). The plant P total uptake was significantly improved with various applied P rates. The optimal uptake of P 0.094 mg pot^{-1} was located in 80 kg P_2O_5 ha^{-1} although 60 kg P_2O_5 ha^{-1} seduced P uptake of 0.060 mg pot^{-1}, while the minimum P uptake of 0.013 mg pot^{-1} was noticed in control. The interactive effect of both PSB strains and different P levels on plant P total uptake was noted as effective in increasing the storing of P in plants. The maximum total P uptake of 0.106 mg pot^{-1} was deduced where *Bacillus PIS7* and 80 kg P_2O_5 ha^{-1} was incorporated after then *Bacillus DUM7* along with 80 kg P_2O_5 ha^{-1} which resulted total P uptake of 0.093 mg pot^{-1}, whereas the minimum total P uptake of 0.011 mg pot^{-1} was observed in *Bacillus CUM6*

without P level was applied.

The current study also demonstrated that PSB strains improve maize plants' uptake of P and that this increased uptake reduced the accumulation of heavy metals and worked as phytoremediation of heavy metals in contaminated soil conditions. Ghosh et al. (2023) recently reported that heavy metals stress on plants caused by the polluted substrate can be alleviated by the phytoremediation of pollutants in conjunction with PSB. In addition, Lai et al. (2023) reported that the process of remediation most likely caused metal phosphate to precipitate or co-precipitate, which resulted in P-induced metal immobilization and increase P uptake by plants.

3.6.2 Plant Cd uptake (mg pot $^{-1}$)

The Cd total uptake was significantly decreased with PSB strains. Among different PSB stains, the maximum Cd total uptake of 5.46 mg pot^{-1} was deduced in *Bacillus PIS7* strain after then *Bacillus DUM7*, resulted Cd total uptake of 5.16 mg pot^{-1} although the lessened Cd total uptake of 4.94 mg pot $^{-1}$ was located in no-supplemented site (Table 4). The use of fertilizers as phosphorous significantly reduced Cd total uptake. Among various P rates, the maximal Cd total uptake of 6.0 mg pot $^{-1}$ was observed where P was applied at 40 kg P_2O_5 ha^{-1} statistically followed by control treatment that attained Cd total uptake of 5.79 mg pot^{-1}, while the minimum Cd total uptake of 3.74 mg pot^{-1} was noticed in 80 kg ha $^{-1}$ P_2O_5 treatment. The combination among PSB strains and various P levels on Cd total uptake was found statistically significant. Among the interaction data, the maximum Cd total uptake of 6.34 mg pot^{-1} was found where *Bacillus PIS7* along with 40 kg ha $^{-1}$ P_2O_5 was used although *Bacillus DUM7* with 40 kg P_2O_5 ha^{-1} that resulted Cd total uptake of 5.97 mg pot^{-1}, although the lessened Cd total uptake of 3.53 mg pot $^{-1}$ was deduced in *Bacillus DUM7* with 80 kg P_2O_5 ha^{-1} supplementation.

The implementation of P efficiently lessened the bioavailable fractions of Cd in soil thus decreasing plant uptake (Cao et al., 2009; Zhu et al., 2015; Liang et al., 2014; Lu et al., 2017).

3.6.3 Plant Pb uptake (mg pot $^{-1}$)

The use of various PSB strains positively reduced Pb total uptake, thus the maximum Pb total uptake of 4.49 and 4.48 mg pot^{-1} were noticed in *Bacillus CUM6* and *Bacillus DUM7* pots, whereas the minimum Pb total uptake of 4.41 mg pot^{-1} was noted in *Bacillus PIS7* (Table 4). The present results also depicted that different P application rates significantly decreased the Pb total uptake. Among different P levels, the maximum Pb total uptake of 5.85 mg pot^{-1} was resulted in control treatment pot followed by 40 kg P_2O_5 ha^{-1} which attained Pb total uptake of 4.66 mg pot^{-1}, while the minimum Pb total uptake of 3.55 mg pot^{-1} was located in 80 kg ha $^{-1}$ P_2O_5 treatment pot. The interaction among PSB strains and several P doses on Pb total uptake was observed statistically significant. The higher Pb total uptake of 5.91 and 5.90 mg pot^{-1} were found where *Bacillus CUM6* and *Bacillus DUM7* with no P application treatments followed by the treatment where only *Bacillus PIS7* was applied that resulted Pb total uptake of 5.75 mg pot^{-1}, although the lessened Pb total uptake of 3.33 mg pot $^{-1}$ was recorded in *Bacillus DUM7* along with

80 kg ha $^{-1}$ P_2O_5 application. The overall findings suggested that the total intake of Pb was dramatically reduced at different P fertilizer doses, and this might be because these heavy metals precipitated or adhered to phosphatic ions. In addition, PSB strains, particularly *Bacillus PIS7* bacteria, significantly reduced the total uptake of Pb.

The results of Sharma and Archana, (2016) were in similarity with the present findings in which various bacterial species reduced the Pb total uptake. It was reported by Hussain et al. (2023) that phosphorous solubilizing bacteria decreased the Pb total uptake. Quezada-Hinojosa et al. (2015) documented that the use of bacterial strains reduced the Pb total uptake. Similarly, Chen et al. (2019) also observed that several bacterial strains reduced the Pb total uptake.

3.6.4 Plant Cr uptake (mg pot $^{-1}$)

Application of various bacterial strains reduced the total uptake of Cr (Marzban et al., 2016; Zhao et al., 2022). The results showed that PSB strains efficiently decreased the Cr total uptake, thus the optimal observations 1.71 mg pot $^{-1}$ was noticed in *Bacillus CUM6* while the minimum outcomes 1.43 mg pot $^{-1}$ was recorded in *Bacillus PIS7* strain (Table 4). The Cr total uptake was significantly reduced with various P levels. The maximum Cr total uptake of 2.17 mg pot^{-1} was noted in control treatment pot followed by 40 kg P_2O_5 ha^{-1} treatment pot resulted Cr total uptake of 1.88 mg pot^{-1} while the minimum Cr total uptake of 0.77 mg pot^{-1} was noticed in 80 kg P_2O_5 ha^{-1} treatment. The interactive effective of both PSB strains and different P levels on plant Cr total uptake was noted effective. The maximum Cr total uptake of 2.19 and 2.17 mg pot^{-1} were found where *Bacillus DUM7* and *Bacillus CUM6* with no P application treatments followed by *Bacillus PIS7* without P application that resulted Cr total uptake of 2.16 mg pot $^{-1}$ whereas although the lessened Cr total uptake of 0.52 mg pot $^{-1}$ was recorded in *Bacillus PIS7* along with 80 kg ha $^{-1}$ P_2O_5 application. The overall findings suggested that the total intake of Cr was dramatically reduced at different P fertilizer doses, and this might be because these heavy metals precipitated or adhered to phosphatic ions. In addition, PSB strains, particularly *Bacillus PIS7* bacteria, significantly reduced the total uptake of Cr.

3.6.5 Plant Ni uptake (mg pot $^{-1}$)

Bacterial strains lowered the total uptake of Ni (Zeng et al., 2017). The Ni bioaccumulation factor was significantly decreased with PSB strains. Among different PSB stains, the maximum Ni total uptake of 2.31 mg pot $^{-1}$ was deduced in *Bacillus CUM6* strain although the lesser Ni total uptake of 2.13 mg pot^{-1} was noted in *Bacillus PIS7* (Table 4). The use of P fertilizers significantly reduced Ni total uptake. Among various P levels, the higher observations 2.82 mg pot $^{-1}$ was noted in control although 40 kg P_2O_5 ha^{-1} that attained Ni total uptake of 2.29 mg pot^{-1}, while the minimum Ni total uptake of 1.75 mg pot^{-1} was noticed in 80 kg P_2O_5 ha^{-1} treatment pot. The interaction among PSB strains and various P levels on Ni total uptake was found statistically significant. Among the interaction data, the maximum Ni total uptake of 2.84 and 2.84 mg pot^{-1} were found in *Bacillus DUM7* and *Bacillus PIS7* with no phosphorous application respectively followed by *Bacillus CUM6* without P fertilizer that resulted Ni total uptake of 2.77

mg pot^{-1}, and the minimum outcomes 1.63 mg pot^{-1} was located in *Bacillus PIS7* with 80 kg ha^{-1} P_2O_5 application. The overall results seduced that the total uptake of Ni was significantly decreased with varying levels of P fertilizer and this might be due to the precipitation or adsorption of these heavy metals with phosphatic ions. Besides this, PSB strains also drastically decreased the total uptake of Ni, especially *Bacillus PIS7* strains.

3.7 Effect of rhizobacteria and applied phosphorous on the heavy metal's translocation

3.7.1 Plant Cd translocation

The use of synthetic P fertilizers significantly inhibits the translocation of Cd (Liu et al., 2020; Mitra et al., 2018; Li et al., 2022). The Cd translocation factor was significantly decreased with PSB strains. Among different PSB stains, the maximum Cd translocation factor of 0.90 and 0.89 was deduced in *Bacillus CUM6* and *Bacillus DUM7* strain whereas the minimum Cd translocation factor of 0.88 was noted in *Bacillus PIS7* (Table 5). The use of synthetic fertilizers as phosphorous significantly reduced Cd translocation factor. Among various P levels, the maximum Cd translocation factor of 0.95 was noted in control although 40 kg P_2O_5 ha^{-1} attained Cd translocation factor of 0.92 while the minimum Cd translocation factor of 0.80 was noticed in 80 kg ha^{-1} P_2O_5 treatment. The combination among PSB strains and various P levels on Cd translocation factor was found statistically significant. Among the interaction data, the maximum Cd translocation factor of 0.97 was found in *Bacillus CUM6* with no phosphorous application that was closely followed by *Bacillus DUM7* and *Bacillus PIS7* with no P fertilizer treatment that resulted in Cd translocation factor of 0.95 and 0.94 whereas the minimum Cd translocation factor of 0.78 was noted in *Bacillus CUM6* with 80 kg ha^{-1} P_2O_5 application.

3.7.2 Plant Pb translocation

The use of various PSB strains positively reduced Pb translocation factor, thus the maximum Pb translocation factor of 0.83 was noticed in *Bacillus CUM6* treatment whereas the minimum Pb translocation factor of 0.80 was noted in *Bacillus PIS7* (Table 5). The present results also depicted that different P application rates significantly decreased the Pb translocation factor. Among different P levels, the maximum Pb translocation factor of 0.89 resulted in the control treatment pot although 60 kg ha^{-1} P_2O_5 attained Pb translocation factor of 0.83 while the minimum Pb translocation factor of 0.75 was located in 80 kg ha^{-1} P_2O_5 treatment. The combination among PSB strains and different P levels on the Pb translocation factor was found statistically significant. The maximum Pb translocation factor of 0.91 was found where *Bacillus CUM6* with no P application treatments that was followed by the treatment where only *Bacillus DUM7* was applied resulted in Pb translocation factor of 0.89, whereas the minimum Pb translocation factor of 0.74 was recorded in *Bacillus PIS7* along with 80 kg P_2O_5 ha^{-1} application.

Phosphate solubilizing bacteria releases phosphates inside soil that makes phosphate mineralizing precipitates with toxic metals that significantly reduce the Pb translocation factor (Xiao et al., 2021). The degree of Pb translocation factor were positively decreased with bacterial strains as it has the potential of

Table 5. Effect of PSB strains and various P levels on plant translocation of P, Cd, Pb, Cr, Ni.

P Levels	PSB Strains	Plant translocation			
		Cd	Pb	Cr	Ni
0	Bacillus CUM6	0.97 a	0.91 a	0.39 a	0.54 a
40	Bacillus CUM6	0.92 c	0.85 d	0.35 d	0.47 b
60	Bacillus CUM6	0.91 e	0.80 f	0.28 g	0.41 d
80	Bacillus CUM6	0.78 i	0.77 g	0.17 j	0.36 h
0	Bacillus DUM7	0.95 b	0.89 b	0.38 b	0.54 a
40	Bacillus DUM7	0.91 d	0.84 d	0.31 e	0.47 c
60	Bacillus DUM7	0.86 f	0.80 f	0.23 h	0.38 f
80	Bacillus DUM7	0.79 h	0.75 h	0.12 k	0.33 i
0	Bacillus PIS7	0.94 b	0.88 c	0.37 c	0.53 a
40	Bacillus PIS7	0.93 c	0.82 e	0.29 f	0.38 f
60	Bacillus PIS7	0.87 f	0.76 g	0.19 i	0.37 g
80	Bacillus PIS7	0.84 g	0.74 h	0.08 l	0.30 j
	SOV				
	PSB strain	*	*	*	*
	P	*	ns	*	**
	PSB*P	ns	ns	ns	ns

Note: . Values in the column followed by the same letters are statistically the same. SOV-Source of variation, P-Phosphorous, ns-non significant, * - significant at 5 % ($P < 0.05$). **- Significant at 1 % ($P < 0.01$).

biological adsorption (Priya et al., 2022). Different rhizospheric bacteria immobilize metals by excreting organic compounds and viscous slime outside the bacterial cell for a variety of purposes, including adherence to surfaces, protection, water retention (Madhaiyan et al., 2007). Phosphate-solubilizing bacteria's processes for immobilizing heavy metals mostly involve heavy metal fixation and the release of soil's available P. (Qin et al., 2023). Furthermore, phosphate-solubilizing bacteria stabilize heavy metals by releasing PO43 to create heavy metal precipitates or by bio-sorption, excreting metabolites to chelate or modify the form of heavy metals, hence lowering their absorption by crops (Marzban et al., 2016). Numerous phosphate-solubilizing bacterial strains considerably decreased the bioavailable proportion of heavy metals in

polluted soil by precipitation and adsorption, which in turn decreased their translocation (Zhao et al., 2022) .

3.7.3 Plant Cr translocation

Phosphate solubilizing bacteria significantly reduced the Cr translocation factor (Sharma and Archana, 2016; Zhang et al., 2021). The PSB strains efficiently decreased the Cr translocation factor, thus the maximum Cr translocation factor of 0.29 was noticed in *Bacillus CUM6* while the minimum Cr translocation factor of 0.23 was recorded in *Bacillus PIS7* strain (Table 5). The Cr translocation factor was significantly reduced with various P levels. The maximum Cr translocation factor of 0.38 was noted in control treatment that was followed by 40 kg P_2O_5 ha^{-1} treatment resulted Cr translocation factor of 0.31, while the minimum Cr translocation factor of 0.12 was noticed in 80 kg P_2O_5 ha^{-1} treatment. The interactive effective of both PSB strains and different P levels on plant Cr translocation factor was noted effective. The maximum Cr translocation factor content of 0.39 was found where *Bacillus CUM6* with no P application treatment that was followed by the treatment where only *Bacillus DUM7* that resulted Cr translocation factor content of 0.38 whereas the minimum Cr translocation factor content of 0.08 was recorded in *Bacillus PIS7* along with 80 kg ha^{-1} P_2O_5 application.

3.7.4 Plant Ni translocation

Bacterial strains reduced the Ni translocation factor as it stabilized the fractions of metals and inhibits their uptake (Liu et al., 2020; Chen et al., 2019). The Ni translocation factor was significantly decreased with PSB strains. Among different PSB stains, the maximum Ni translocation factor of 0.44 was deduced in *Bacillus CUM6* strain whereas the minimum Ni translocation factor of 0.40 was noted in *Bacillus PIS7* (Table 5). The use of synthetic fertilizers as phosphorous significantly reduced Ni translocation factor. Among various P levels, the maximum Ni translocation factor of 0.54 was noted in control although 40 kg P_2O_5 ha^{-1} attained Ni translocation factor of 0.45, while the minimum Ni translocation factor of 0.33 was noticed in 80 kg ha^{-1} P_2O_5 treatment. The combination among PSB strains and various P levels over Ni translocation factor was found statistically significant. Among the interaction data, the maximum Ni translocation factor of 0.54 and 0.54 were found in *Bacillus CUM6* and *Bacillus DUM7* with no phosphorous application that was closely followed by *Bacillus CUM6* and *Bacillus DUM7* with 40 kg P_2O_5 ha^{-1} fertilizer treatments that resulted Ni translocation factor of 0.47 and 0.47, whereas the minimum Ni translocation factor of 0.30 was noted in *Bacillus PIS7* with 80 kg P_2O_5 ha^{-1} supplementation. Reduced availability of heavy metals was associated with how distinct inorganic phosphorus forms were affected by phosphorus-dissolving bacteria (Liu et al., 2020).

4. Conclusion

The combined implementation of PSB strain *Bacillus PIS7* with 80 kg P_2O_5 ha^{-1} significantly improved plant growth (fresh and dry biomass) under stressful environments compared to the control group without spiked heavy metals in the soil. P bioavailability was dramatically increased with the application of P fertilizer (80 kg ha^{-1}) and PSB strains, particularly *Bacillus PIS7.* The fraction of heavy metals (Cd, Pb, Cr, and

Ni) in both soil and plants was significantly reduced with the application of PSB strains and P fertilizer, with 80 kg P_2O_5 ha $^{-1}$ and *Bacillus PIS7* showing superior performance. The total uptake of P increased linearly with varying levels of applied P along with PSB strains, closely paralleling maize growth response. Among the tested PSB strains, *Bacillus PIS7* with 80 kg P_2O_5 ha $^{-1}$ exhibited the minimum translocation of heavy metals compared to the control and other PSB strains. Organic matter content increased linearly, while soil pH and electrical conductivity decreased with applied phosphorus and PSB strains. Overall, rhizobacteria utilization, especially *Bacillus PIS7*, played a significant role in enhancing plant resistance to harmful heavy metals (Cd, Pb, Cr, and Ni) and regenerating soil health. The application of 80 kg P_2O_5 ha $^{-1}$ with *Bacillus PIS7* is recommended to achieve better growth response under heavy metal stress and stabilize Cd, Pb, Cr, and Ni. Further experiments are needed to explore the potential of different PSB strains combined with P fertilizers for improved plant growth and heavy metal stabilization.

Acknowledgments:

The authors are thankful to the Department of Soil and Environmental Sciences Faculty of Crop Production Sciences, The University of Agriculture, Peshawar-Pakistant for providing all the necessary facilities required to perform this research work.

Conflicts of Interest: The authors declare no conflict of interest.

Availability of Data and Materials: Data will be available on demand from the corresponding authors.

Authors Contributions

AN, and HN, convinced the main idea. AN, and HQ., designed the experiment, wrote the manuscript and revised the manuscript. All authors helped in data collection, data analysis and revising the manuscript. The Author (s) read and approved the final manuscript.

REFERENCES

Alsafran, M., Saleem, M. H., Al Jabri, H., Rizwan, M., & Usman, K. Principles and applicability of integrated remediation strategies for heavy metal Removal/Recovery from contaminated environments. Journal of Plant Growth Regulation. (2023). 42(6), 3419-3440.

Ao, X., X. H. Guo, Q. Zhu, H. J. Zhang, H. Y. Wang, Z. H. Ma, X. R. Han, M. H. Zhao and F. T. Xie. Effect of phosphorus fertilization to P uptake and dry matter accumulation in soybean with different P efficiencies. Journal of Integrative Agriculture. (2014).13: 326–334.

Asensio, V., Vega, F. A., Singh, B. R., & Covelo, E. F. Effects of tree vegetation and waste amendments on the fractionation of Cr, Cu, Ni, Pb and Zn in polluted mine soils. Science of the total environment. (2013). 443, 446-453.

Bhatt, M. K., K. P. Raverkar, R. Labanya and C. K. Bhatt. Effects of long-term balanced and imbalanced use of inorganic fertilizers and organic manure (FYM) on soil chemical properties and yield of rice under rice-wheat cropping system. Journal of Pharmacognosy and Phytochemistry. (2018).7(3): 703-708.

Bremner, J. M., & Mulvaney, C. S. Nitrogen—total. Methods of soil analysis: part 2 chemical and microbiological properties. (1983). 9, 595-624.

Cao X., A. Wahbi, L. Ma, B. Li and Y. Yang. Immobilization of Zn, Cu, and Pb in contaminated soils using phosphate rock and phosphoric acid. Journal of Hazardous Materials. (2009).164(2-3): 555.

Chen, H., J. Zhang, L. Tang, M. Su, D. Tian, L. Zhang, Z. Li and S. Hu. Enhanced Pb immobilization via the combination of biochar and phosphate solubilizing bacteria. Environment international. (2019).127: 395-401.

Delorme, T., Gagliardi J, Angle J, Influence of the zinc hyperaccumulator Thlaspi caerulescens J. & C. Presl. and the nonmetal accumulator Trifolium pratense L. on soil microbial populations. Canadian Journal of Microbiology. (2001). 47: 773–776.

Dey, G., Banerjee, P., Sharma, R. K., Maity, J. P., Etesami, H., Shaw, A. K., Chen, C. Y. Management of phosphorus in salinity-stressed agriculture for sustainable crop production by salt-tolerant phosphate-solubilizing bacteria—A review. Agronomy. (2021). 11(8), 1552.

El Sayed, H. E., Othaimen, H. S., Aburas, M. M., & Jastaniah, S. D. Efficiency of an Cd-tolerant actinomycete isolate obtained from wastewater in removal of heavy metals and enhancing plant growth of Zea mays L. plant. Int J Curr Microbiol Appl Sci. (2015). 4, 553-565.

El-Meihy, R. M., H. E. Abou-Aly, T. A. Tewfike, E. A. El-Alkshar and A. M. Youssef. Characterization and identification of cadmium-tolerant bacteria isolated from contaminated regions in Egypt. Biocatalysis and Agricultural Biotechnology. (2019). 21(10)12–99.

Fahsi, N., I. Mahdi, A. Mesfioui, L. Biskri and A. Allaoui. Plant Growth-Promoting Rhizobacteria isolated from the Jujube (Ziziphus lotus) plant enhance wheat growth, Zn uptake, and heavy metal tolerance. Agriculture. (2021).11(4): 316.

Falamaki A., H. Tavallali, M. Eskandari and S. R. Farahmand. Immobilizing some heavy metals by mixing contaminated soils with phosphate admixtures. International Journal of Civil Engineering. (2016).14 (2): 75.

Feng, N. X., Yu, J., Zhao, H. M., Cheng, Y. T., Mo, C. H., Cai, Q. Y., Wong, M. H. Efficient phytoremediation of organic contaminants in soils using plant–endophyte partnerships. Science of the Total Environment. (2017). 583, 352-368.

Gadd, G. M. Microbial influence on metal mobility and application for bioremediation. Geoderma. (2004). 122(2-4), 109-119.

Ghosh, D., B. M. Singh, M. Kumar, S. K. Maiti and N. K. Dhal. Role of Endophytic Microorganisms in Phosphate Solubilization and Phytoremediation of Degraded Soils. In Plant Microbiome for Plant Productivity and Sustainable Agriculture. (2023).387-400.

Hashimoto Y., M. Takaoka, K. Oshita and H. Tanida. Incomplete transformations of Pb to pyromorphite by phosphate-induced immobilization investigated by X-ray absorption fine structure (XAFS) spectroscopy. (2009).76(5): 616.

Huang G., X. Su, M. S. Rizwan, Y. Zhu and H. Hu. Chemical immobilization of Pb, Cu, and Cd by phosphate materials and calcium carbonate in contaminated soils. Environmental Science and Pollution Research. (2016). 23(16):1.

Hussain, A., M. Shah, M. Hamayun, A. Iqbal, M. Qadir, A. Alataway, A. Z. Dewidar, H. O. Elansary and I.-J. Lee. Phytohormones producing rhizobacteria alleviate heavy metals stress in soybean through multilayered

response. Microbiological Research, (2023). 266: 127-237.

Janeena, A., V. Nagabalaji, P. Suresh, K. N. Ramudu, S. V. Srinivasan, G. Shanmugam and N. Ayyadurai. Engineering microbial cells with metal chelating hydroxylated unnatural amino acids for removable of synthetic pollutants from water. Chemosphere. (2023). 311: 13:67-56.

Kamal, S., Prasad, R., & Varma, A. Soil microbial diversity in relation to heavy metals. Soil heavy metals. (2010). 31-63.

Kesari, K. K., Soni, R., Jamal, Q. M. S., Tripathi, P., Lal, J. A., Jha, N. K., ... & Ruokolainen, J. Wastewater treatment and reuse: a review of its applications and health implications. Water, Air, & Soil Pollution. (2021). 232, 1-28.

Khalid, S., Shahid, M., Niazi, N. K., Murtaza, B., Bibi, I., & Dumat, C. A comparison of technologies for remediation of heavy metal contaminated soils. Journal of geochemical exploration. (2017). 182, 247-268.

Khanna, K., Jamwal, V.L., Gandhi, S.G., Ohri, P. and Bhardwaj, R., Metal resistant PGPR lowered Cd uptake and expression of metal transporter genes with improved growth and photosynthetic pigments in Lycopersicon esculentum under metal toxicity. Scientific reports. (2019). 9(1)1-14.

Khanna, K., Kohli, S. K., Ohri, P., Bhardwaj, R., Al-Huqail, A. A., Siddiqui, M. H., ... & Ahmad, P. Microbial fortification improved photosynthetic efficiency and secondary metabolism in Lycopersicon esculentum plants under Cd stress. Biomolecules.(2019). 9(10), 581.

Lai, H. J., Ding, X. Z., Cui, M. J., Zheng, J. J., Chen, Z. B., Pei, J. L., & Zhang, J. W. Mechanisms and Influencing Factors of Biomineralization Based Heavy Metal Remediation: a Review. Biogeotechnics. (2023). 100039.

Li, Q., and Y. Gao. Remediation of Cd-, Pb- and Cu-contaminated agricultural soils by phosphate fertilization and applying biochar. Polish Journal of Environmental Studies, (2019). 28(4): 2697-2705.

Liang Y., X. Cao, L. Zhao and E. Arellano. Biocharand phosphate-induced immobilization of heavy metals in contaminated soil and water: Implication on simultaneous remediation of contaminated soil and groundwater. Environmental Science Pollution Research. (2014). 21(6): 4665.

Liu, M., Z. Zhao, L. Chen, L. Wang, L. Ji and Y. Xiao. Influences of arbuscular mycorrhizae, phosphorus fertiliser and biochar on alfalfa growth, nutrient status and cadmium uptake-sciencedirect.
Ecotoxicology and Environmental Safety (2020). 196: 110537.

Lu H.P., Z. A. Li, G. Gascó, A. Méndez, Y. Shen, J. Paz-Ferreiro. Use of magnetic biochars for the immobilization of heavy metals in a multi-contaminated soil. Science Total Environment. (2017). 622-623: 892.

Madhaiyan, M., Poonguzhali, S., Lee, J. S., Lee, K. C., & Hari, K. Bacillus rhizosphaerae sp. nov., an novel diazotrophic bacterium isolated from sugarcane rhizosphere soil. Antonie van Leeuwenhoek. (2011). 100, 437-444.

Manasa, R. L., & Mehta, A. Wastewater: sources of pollutants and its remediation. Environmental Biotechnolog. (2020).Vol. 2, 197-219.

Marzban, A., Ebrahimipour, G., Karkhane, M., & Teymouri, M. Metal resistant and phosphate solubilizing bacterium improves maize (Zea mays) growth and mitigates metal

accumulation in plant. Biocatalysis and Agricultural Biotechnology. (2016). 8, 13-17.

Mehrotra, T., S. Dev, A. Banerjee, A. Chatterjee, R. Singh and S. Aggarwal. Use of immobilized bacteria for environmental bioremediation: A review. Journal of Environmental Chemical Engineering. (2021). 9(5):10:9-20.

Mendoza-Labrador, J., F. Romero-Perdomo, J. Abril, J. P. Hernández, D. Uribe-Vélez and R. B. Buitrago. Bacillus strains immobilized in alginate macrobeads enhance drought stress adaptation of Guinea grass. Rhizosphere (2021).19:100– 385.

Mengoni, A., Barzanti, R., Gonnelli, C., et al. Characterization of nickel-resistant bacteria isolated from serpentine soil. Environmental Microbiology. (2001). 3:691–698.

Mohammed, A. S., Kapri, A., & Goel, R. Heavy metal pollution: source, impact, and remedies. Biomanagement of metal-contaminated soils. (2011). 1-28.

Moreno-Galván, A., F. A. Romero-Perdomo, G. Estrada-Bonilla, C. E. Salvino and R. R. Bonilla. Dry-caribbean Bacillus spp. strains ameliorate drought stress in maize by a strain-specific antioxidant response modulation. Microorganisms. (2020).8:8-23.

Muhammad, N., Nafees, M., Khan, M. H., Ge, L., & Lisak, G. (2020). Effect of biochars on bioaccumulation and human health risks of potentially toxic elements in wheat (Triticum aestivum L.) cultivated on industrially contaminated soil. Environmental Pollution, 260, 113887.

Nadeem, N., Asif, R., Ayyub, S., Salman, S., Shafique, F., Ali, Q. and Malik, A., 2020. Role of rhizobacteria in phytoremediation of heavy metals. Biological and Clinical Sciences Research Journal. (2020):(1).

Naeem, A., M. Aslam and A. Lodhi. Improved potassium nutrition retrieves phosphorus-induced decrease in zinc uptake and seed zinc concentration of wheat. Journal of Science Food Agriculture. (2018). 98: 4351–4356.

Nelson, D. W., & Sommers, L. E. Total carbon, organic carbon, and organic matter. Methods of soil analysis: Part 3 Chemical methods .(1996). 5, 961-1010.

Pal, B., S. Pati, P. K. Patra and S. Badole. Effect of integrated nutrient management on nitrogen dynamics in the soil of rice-potato based cropping system. Journal of Applied and Natural Science. (2007). 7(2): 652-655.

Pratush, A., Kumar, A., & Hu, Z. (2018). Adverse effect of heavy metals (As, Pb, Hg, and Cr) on health and their bioremediation strategies: a review. International Microbiology, 21, 97-106.

Priya, A., L. Gnanasekaran, K. Dutta, S. Rajendran, D. Balakrishnan and M. Soto-Moscoso. Biosorption of heavy metals by microorganisms: Evaluation of different underlying mechanisms. Chemosphere. (2022). 307: 135:9-57.

Quezada-Hinojosa, R., K. B. Föllmi and F. Gillet. Cadmium accumulation in six common plant species associated with soils containing high geogenic cadmium concentrations at Le Gurnigel, Swiss Jura Mountains. Catena. (2015).124: 85-96.

Raymond, N. S., B. Gómez-Muñoz, F. J. Van-der-Bom, O. Nybroe, L. S. Jensen, D. S. Müller-Stöver, A. Oberson and A. E. Richardson. Phosphate-solubilising microorganisms for improved crop productivity: a critical assessment. New Phytol. (2021). 229:1268–1277.

Rizvi, A., Ahmed, B., Khan, M. S., Rajput, V. D., Umar, S., Minkina, T., & Lee, J. Maize

associated bacterial microbiome linked mitigation of heavy metal stress: a multidimensional detoxification approach. Environmental and Experimental Botany. (2022). 200, 10491.

Rizwan M. S., M. Imtiaz, G. Huang, M. A. Chhajro, Y. Liu, Q. Fu, J. Zhu, M. Ashraf, M. Zafar and S. Bashir. Immobilization of Pb and Cu in polluted soil by superphosphate, multi-walled carbon nanotube, rice straw and its derived biochar. Environmental Science Pollution Reasearch. (2016).23(15): 15:5-32.

Sardar, K., Ali, S., Hameed, S., Afzal, S., Fatima, S., Shakoor, M. B., ... & Tauqeer, H. M. Heavy metals contamination and what are the impacts on living organisms. Greener Journal of Environmental management and public safety. (2013). 2(4), 172-179.

Sarwar, N., S. M. Saifullah, S. S. Malhi, M. H. Zia, A. Naeem, S. Bibia and G. Farida. Role of mineral nutrition in minimizing cadmium accumulation by plants. Journal of Science Food Agriculture. (2010). 90: 925–937.

Sharma, R. K., and G. Archana. Cadmium minimization in food crops by cadmium resistant plant growth promoting rhizobacteria. Applied Soil Ecology. (2016).107: 66-78.

Shazma, A., K. Iftikhar, H. Saddam, M. M. Anjum, I. Babar, H. Ashraf, and A. Nawab. Wheat response to different levels of humic acid and brassinolide. Pure and Applied Biology. (2016). 5(4): 822-829.

Sheoran, V., Sheoran, A., Poonia, P., Role of hyperaccumulators in phytoextraction of metals from contaminated mining sites. Critical Reviews in Environmental Science and Technology. (2011). 41, 168–214.

Soltanpour, P. N., & Schwab, A. P. A new soil test for simultaneous extraction of macro-and micro-nutrients in alkaline soils. Communications in soil science and plant analysis. (1977). 8(3), 195-207.

Teng, Z., Shao, W., Zhang, K., Huo, Y., & Li, M. (2019). Characterization of phosphate solubilizing bacteria isolated from heavy metal contaminated soils and their potential for lead immobilization. Journal of Environmental Management, 231, 189-197.

Tóth, G., Hermann, T., Da Silva, M. R., & Montanarella, L. J. E. I. (2016). Heavy metals in agricultural soils of the European Union with implications for food safety. Environment international, 88, 299-309.

Volpe, M. G., M. Nazzaro, M. Di-Stasio and Siano. Content of micronutrients, mineral and trace elements in some Mediterranean spontaneous edible herbs. Chemistry Central Journal. (2015). 9: 1-9.

Wahid, F., Sharif, M., Steinkellner, S., Khan, M. A., Marwat, K. B., & Khan, S. A. Inoculation of arbuscular mycorrhizal fungi and phosphate solubilizing bacteria in the presence of rock phosphate improves phosphorus uptake and growth of maize. Pakistan Journal of Botany (2016).48(2), 739-747.

Wang, H., S. C. Zhao, R. L. Liu, W. Zhou and J. Y. Jin. Changes of photosynthetic activities of maize (*Zea mays* L.) seedlings in response to cadmium stress. Photosynthetica. (2009). 47; 277–283.

Wang, X., D. Cai, M. Ji, Z. Chen, L. Yao and H. Han. Isolation of heavy metal-immobilizing and plant growth-promoting bacteria and their potential in reducing Cd and Pb uptake in water spinach. Science of the Total Environment. (2022). 819: 15:32–42.

Wuana, R.A., Okieimen, F.E., Heavy metals in contaminated soils: a review of sources, chemistry, risks and best available strategies for remediation. ISRN Ecology. (2011). 1–20.

Xiao, C., S. Guo, Q. Wang and R. Chi. Enhanced reduction of lead bioavailability in phosphate mining wasteland soil by a phosphate-solubilizing strain of Pseudomonas sp., LA, coupled with ryegrass (Lolium perenne L.) and sonchus (Sonchus oleraceus L.). Environmental Pollution. (2021). 274: 11:65–72.

Xu, J. C., Huang, L. M., Chen, C., Wang, J., & Long, X. X. (2019). Effective lead immobilization by phosphate rock solubilization mediated by phosphate rock amendment and phosphate solubilizing bacteria. Chemosphere, 237, 124540.

Yan, A., Wang, Y., Tan, S. N., Mohd Yusof, M. L., Ghosh, S., & Chen, Z.Phytoremediation: a promising approach for revegetation of heavy metal-polluted land. Frontiers in Plant Science. (2020). 11, 359.

Zafar-ul-Hye, M., M. Naeem, S. Danish, S. Fahad, R. Datta, M. Abbas and M. Nasir. Alleviation of cadmium adverse effects by improving nutrients uptake in bitter gourd through cadmium tolerant rhizobacteria. Environments. (2020). 7(8): 54.

Zeng, G., J. Wan, D. Huang, L. Hu, C. Huang, M. Cheng, W. Xue, X. Gong, R. Wang and D. Jiang. Precipitation, adsorption and rhizosphere effect: the mechanisms for phosphate-induced pb immobilization in soils—A review. J. Hazard. Mater. (2017). 339: 354-367.

Zhang, K., D. Zhang, X. Wu and Y. Xue. Continuous and efficient immobilization of heavy metals by phosphate-mineralized bacterial consortium. Journal of Hazardous Materials. (2021). 416: 125-800.

Zhao, X., J. Dai, Z. Teng, J. Yuan, G. Wang, W. Luo, X. Ji, W. Hu and M. Li. Immobilization of cadmium in river sediment using phosphate solubilizing bacteria coupled with biochar-supported nano-hydroxyapatite. Journal of cleaner Production. (2022). 348: 13:12-21.

Zheljazkov, V. D., & McNeil, P. (2008). Comparison of five digestion procedures for recovery of nutrients and trace elements in plant tissue. Journal of Plant Nutrition, 31(11), 1937-1946.

Zhu J., Z. Cai, X. Su, Q. Fu, Y. Liu, Q. Huang, A. Violante and H. Hu. Immobilization and phytotoxicity of Pb in contaminated soil amended with γ-polyglutamic acid, phosphate rock, and γ-polyglutamic acid-activated phosphate rock. Environmental Science Pollution Reasearch. (2015). 22(4): 26-61.

Xu, J. C., Huang, L. M., Chen, C., Wang, J., & Long, X. X. (2019). Effective lead immobilization by phosphate rock solubilization mediated by phosphate rock amendment and phosphate solubilizing bacteria. Chemosphere, 237, 124540.

Yan, A., Wang, Y., Tan, S. N., Mohd Yusof, M. L., Ghosh, S., & Chen, Z. Phytoremediation: a promising approach for revegetation of heavy metal-polluted land. Frontiers in Plant Science. (2020). 11, 359.

How to cite this article:

Nawaz, A., Nawaz, H., Khan, K., Haq, M.U., Khan, H., Mananman, U., Tariq, M. Integrated Effect of Heavy Metal-Tolerant Rhizobacteria and Phosphorus on Maize Growth and Phosphorus Bioavailability in Contaminated Soil. Journal of Soil, Plant and Environment (2023); 2(1)-pp; 21-52